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SOIL SCIENCE

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)IL SCIENCE

VOLUME VII

JANUARY—JUNE, 1919

SOIL SCIENCE

A MONTHLY JOURNAL DEVOTED TO PROBLEMS IN SOIL PHYSICS, SOIL CHEMISTRY AND SOIL BIOLOGY

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ERRATA

VOLUME VI

Page 200, next to last sentence on page, "In this work it is assumed that the *Azotobacter* not only fix the nitrogen but also produce the nitrates," should read: "In the work of Headden it is assumed that the *Azotobacter* not only fix the nitrogen but also produce the nitrates."

Page 401, second line below table 2, and page 402, line 2, "genii" should read "genera."

VOLUME VII

Page 315, legend of figure 2, "Widstoe" should read "Widtsoe."

Page 319, fifth line from foot of page, "Alway" should read "Alway and McDole."

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It is a pleasure to announce that in a short time we expect to have overcome all difficulties, naturally incident to the past year, connected with the editing, publishing and manufacturing of this publication, and to forward promptly at regular intervals all issues in continuation.

THE PUBLISHERS.

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

JAMES JOHNSON

Department of Horticulture, Wisconsin Agricultural Experiment Station

Received for publication January 6, 1918

INTRODUCTION

The use of heat-sterilized soils in methods of research in soil biology and plant pathology as well as in various phases of practical agriculture is rapidly increasing in value. For purposes of research especially, the sterilization of soil by heat is practically the only method which can be relied upon for purposes of rendering the soil sterile as regards any particular organism. Since the application of heat to the soil may, and usually does, result in important changes aside from sterilization which may greatly influence plant growth, it is evident that a knowledge of the alterations which occur or may be expected to occur in the soil is essential in order to draw reliable conclusions from certain types of research conducted with heated soils. The problem is by no means a new one. A great variety of literature exists upon the subject of soil sterilization from various angles of attack. Since the subject may be treated from the standpoint of the soil itself as chemistry, biology, or physics, or from the standpoint of plants grown in these soils as physiology or pathology, the field has been a very productive one. At the same time, its complexity has led to quite widely varying results and conclusions. Although it is evident that investigations in this field should take into account all factors which may be involved before drawing far-reaching conclusions, it is also evident that it is exceedingly difficult to grasp the significance of, and lay proportionate weight upon all the factors concerned. The results presented in this paper were first undertaken from a phytopathological point of view. The bearing of the conclusions drawn on phytopathological research are contemplated in another paper. It is the purpose of this paper to present the data obtained on the changes produced in heated soils as measured by their influence on seed germination and plant growth, and to discuss the probable nature of these changes.

A clear conception of the subject of soil sterilization requires at the outset a good understanding of the methods by which soils may be sterilized. These are: (a) Sterilization by heat, and (b) sterilization by chemicals. The former may again be divided into methods using (a) live steam, and (b) dry heat; the latter into methods using (a) volatile antiseptics, and (b) non-volatile antiseptics. Clearly these methods may be expected to yield funda-

mentally different results, not only in regard to the method employed, but also in regard to the intensity with which it is applied. Technically the term sterilization means the complete destruction of all living matter in a medium, and implies the continued sterility of this medium if desired. This term as used in connection with the treatment of the soil, however, does not necessarily mean the complete destruction of all living matter in the soil nor its continued sterility. Ordinarily it refers to the destruction of one or more specific organisms which are not desired in the soil with no special precautions to prevent reinfestation. In a broader sense, however, the object of soil sterilization is the production of a more favorable medium for the growth of cultivated plants by means of heat or chemical disinfection. This may be brought about as much by the improvement of the chemical, physical, or biological relationships in the soil, as by the simple destruction of one or more forms of undesirable organisms. In view of these facts the terms "partial sterilization" and "pasteurization" have come to be used by some investigators.

The object of soil sterilization in practical agriculture is, therefore, usually either the destruction of some particular plant parasite harbored in the soil, or the improvement of the fertility of the soil by affecting its chemical, biological or physical condition. Where it is practiced for the former reason, the latter results are naturally also secured as secondary effects. Many farmers are, for instance, coming to regard the process as valuable from the standpoint of destruction of weed seeds in plant beds alone.

Various methods of application have been devised with the result that the practice has been increasing rapidly in certain forms of intensive plant culture. Up to the present time, soil sterilization has been found most useful and has received its greatest impetus in the culture of plants under glass. The vegetable forcing house industry particularly, has used the method on a large scale. Florists use it less commonly, but find it advantageous for certain plants and for starting seed. In the culture of tobacco, ginseng, coniferous seedlings, and most seedlings for the market gardener, it has been found well worthy of use.

BRIEF SUMMARY OF LITERATURE

The beneficial action of heated soils upon plant growth was observed long before the sterilizing value of heat was known. According to Sir Humphrey Davy (12) the improvement of "sterile" lands by burning was known to the ancient Romans, the custom having been mentioned by Virgil in his first book of the Georgics. It is well known that the burning of various types of soil became quite general in Europe during the eighteenth century. This method was accompanied by a considerable amount of scientific investigation upon the subject, until the practice fell into disuse about the middle of the last century.

The actual use of sterilizing agents upon the soil for the primary purpose of destroying injurious forms of living organisms present in the soil is largely a

development of the past three or four decades. Accompanying these studies and practices of soil sterilization for the destruction of soil organisms, there has accumulated a great deal of literature dealing with the scientific principles involved. These publications have been especially stimulated by a desire to explain the reason for the increased growth of plants on sterilized soils. The investigations undertaken from quite different angles of attack and with great variation in type of soil, kind and intensity of sterilizing agents, as well as in type of plants used, have quite naturally resulted in widely varying conclusions.

A detailed review of the literature concerning soil sterilization would be too voluminous to present here. For present purposes it may suffice to present in brief form an outline of the principal changes produced in and by sterilized soils, and to mention briefly certain theories concerning the nature of the action of sterilized soils on plant growth, leaving the discussion of the literature more directly concerned with this investigation to be treated under the separate phases of the subject as they are taken up.

Principal changes produced in and by sterilized soils.

I. Destruction of life.
   - A. *Normal soil flora and fauna*, desirable and undesirable forms of bacteria, fungi, protozoa, and higher animals.
   - B. *Plant parasites*, especially pathogenic bacteria, fungi, nematodes, and injurious soil infesting insects.
   - C. *Propagative organs of higher plants*, especially weed seeds.

II. Immediate chemical action (formation of toxic and beneficial compounds).
   - A. *Decomposition of organic material* resulting in formation of ammonia, carbon dioxide and various new and complex organic compounds.
   - B. *Decomposition of inorganic material*, reduction of nitrates and nitrites to ammonia and increased solubility of potassium, phosphorus and other salts.

III. Bio-chemical action.
   - A. *Increased ammonification* particularly and modified nitrification, denitrification, and nitrogen fixation.

IV. Physical action.
   - A. *Absorptive capacity of soil* modified for water, gases, and salts.
   - B. *Increased concentration of soil solution.*
   - C. Modified capillarity, colloidal state, and mechanical condition.
   - D. Modified color and odor.

V. Action on organisms growing in sterilized soils.
   - A. Lower organisms.
     1. Increased development due to reduced competition, increased food supply, destruction of "bacterio-toxins," "stimulation" by products added or formed, or other causes.
     2. Retardation in growth in rare cases due to injurious conditions produced.
   - B. Green plants.
     1. Injurious action as indicated by retarded rate and percentage of seed germination and by retarded rate of plant growth.
     2. Beneficial action as shown by increased rate and percentage of seed germination and increased rate and amount of plant growth.
     3. Modification in form, color, and other "qualitative" changes.

The theories which have been promulgated by various investigators rest primarily upon the explanation of the beneficial and injurious action of sterilized soils upon plant growth. These theories are partially interrelated and do not permit of satisfactory classification, but the main ones may be grouped as follows:

1. Stimulation hypothesis. Koch's (31) theory formulated in 1899 was probably the first attempt at scientific explanation of observed facts. He believed the sterilizing agent or its products directly "stimulated" the growth of the plants or soil bacteria, which in turn influenced plant growth.

2. Modified bacterial activity. Hiltner and Störmer (25) in 1903 showed by soil bacterial counts that sterilization resulted in an initial decrease in numbers of organisms followed by a marked increase in numbers, and hence an increase in the productiveness of the soil.

3. Protozoan theory. Russell and Hutchinson (62) believe that the benefit from "partial sterilization" may result from the destruction of the larger phagocytic microörganisms (mostly protozoans) which inhibit the development of the beneficial organisms. The protozoa being destroyed, the ammonifying bacteria, for instance, increase rapidly producing an increase in nitrogen and hence plant growth.

4. "Bacterio-toxin" theory. Grieg-Smith (22) considers that certain substances which he calls "bacterio-toxins" and which exist naturally in soils, inhibiting bacterial growth, are destroyed by sterilizing agents.

5. Modified organic soil compounds. Schreiner and Lathrop (65) believed that certain complex organic soil constituents produced as a result of sterilization by heat, may increase plant growth while others inhibit it. That organic soil constituents of an indefinite nature were produced which were injurious to plant growth in heated soils had also been suggested by Struckman (73), Dietrich (13) and Pickering (52).

6. Modified inorganic soil compounds. This theory though supported by no investigators in particular should be added here since it has been repeatedly shown since the time of Struckman (73) that increase in inorganic plant food constituents occurs in heated soils. The theory is perhaps best supported by Liebscher (40) who believed that sterilization was essentially nitrogenous fertilization.

7. Plant parasite theories. The fact that in certain soils the benefit of soil sterilization may be due largely to the destruction of parasitic organisms is unquestionable. The wide application of this theory, however, to the subject in question, as supported perhaps most energetically by Bolley (5), serves to place this type of response to sterilization among the theories explaining the action of sterilized soils.

8. Physical theories. These theories are not subscribed to by any author in particular at the present time, although it was quite generally believed at one time that all the benefit derived from burning the soil was due to purely physical changes. Some of the physical factors which play a part in soil fer-

tility are, however, coming to be regarded as very influential in conjunction with chemical factors. Seaver and Clark's (67) "concentration theory" may, for instance, properly be placed here.

For a more detailed summary of the subject of soil sterilization especially as regards the relation of protozoa to sterilized soils, the recent review of Kopeloff and Coleman (34) should be consulted.

EXPERIMENTAL WORK

The investigations upon the subject of soil sterilization were begun by the writer in 1909 with the object of studying methods for the control of the damping-off disease of plants, caused by *Pythium debaryanum*, and *Rhizoctonia*. At the outset, it became evident that when soils were treated either with heat or chemicals, various "secondary effects" of sterilization occurred, which modified the soil and plant growth aside from the control of disease. Following a publication upon the control of damping-off in plant beds (27), the study of these "secondary effects" was undertaken. The primary object of this study was to attempt to find an explanation for the injurious action followed by the beneficial action of sterilized soils on plant growth. On account of the size and complexity of the problem, it became necessary to limit the investigation primarily to the effects of sterilization by heat. The studies up to date have been mainly concerned with the action of heated soils upon seed germination and plant growth; although various other phases have been taken up from time to time with the general idea of rounding out the problem in such a way as to make it of value also in the plant pathological investigations carried on simultaneously. In carrying on this work the soil has in many cases been heated to a degree far above that which is used in ordinary sterilization. While no apology may be necessary for such procedure from a chemical standpoint, the general idea has been to increase the action so that there would be produced a sufficient magnification of effect to obtain a good theoretical working basis for the explanation of similar results secured at ordinary temperatures of sterilization. While comparisons cannot readily be made with the "partial sterilization" of Russell and his associates, (62, 64), yet it is hoped that some evidence of value in this connection may be gathered from the results. The work presented here is perhaps more comparable to that of Pickering (50–53), or Seaver and Clark (67, 68), than to that of other investigators. On the other hand the writer has gone into the subject with a desire to bring together the various phases of the subject as presented in the literature with the idea of rounding out the problem and attempting to clear up some of the obscure points.

Materials and methods

The different soils used were selected largely for their variations in general type, especially in physical structure, rather than for differences in their chemi-

cal properties or their productivity. Their names will, therefore, quite satisfactorily indicate the general nature of these soils. No complete mechanical analyses have been made, but a determination of the loss on ignition indicates the range of organic matter present in the various soils as well as the general character of each soil in regard to this constituent. The determinations of total nitrogen, phosphorus, and potassium serve to give an idea of the state of fertility of these soils as far as these elements are concerned. The general character of the soils used is summarized in table 1.[1]

TABLE 1

General character of soils used in the experimental work

NAME	LOSS ON IGNITION	N	P_2O_5	K_2O	SOURCE	GENERAL DESCRIPTION
	per cent	*per cent*	*per cent*	*per cent*		
Waukesha silt loam.....	5.4	0.26	0.056	1.65	Milton, Wisconsin	Dark, tillable, fairly productive
Muck.....	14.8	0.65	0.14	1.52	Station farm, Madison	From drained cropped land, fairly fertile but responding to nitrogen
Virgin sandy loam.....	5.6	0.26	0.054	0.66	Station farm, Madison	Dark, good texture, not fertile from wooded pasture land
Miami silt loam.....		0.20	0.07	1.69	Station farm, Madison	From field cropped 10 years to tobacco but heavily fertilized
Fine sandy loam.....	4.3	0.14	0.068	1.20	Station orchard, Madison	A medium light soil containing considerable silt, not very productive
Peat.....	68.1	2.82			Station farm, Madison	A thoroughly decomposed peat, previously cropped and quite fertile
Norfolk sand.....	1.9	0.05			Upper Marlboro, Maryland	A very light sand, relatively low in productivity
Red clay.....	5.5	0.02			Ashland, Wisconsin	Very heavy red clay and non-productive

The soils were taken from the fields during the fall and allowed to air dry for most of the work where dry heat was used. The methods of heating the soils were usually of two kinds: First by steam in the autoclave at which a temperature of 114°-116°C. was maintained for one and one-half hour, or second by dry heat, in gas, electric, or hot water ovens. For temperatures of 500°C. or

[1] The writer is indebted to the Soils Department, University of Wisconsin, for the determinations of nitrogen, potassium, and phosphorus.

over the electric furnace was used. The time of heating was usually brought as near to two hours as possible.

When the work was first started and in fact during the greater part of this investigation, it was supposed that the time of heating the soil was of comparatively small consequence. The soil was usually, therefore, kept at the desired temperature for only a comparatively short period of time, the heat being allowed to increase gradually in the dry ovens for the first hour. Later on it became evident that a source of experimental error lay in the length of time of heating and the lack of uniformity of heat distribution in large samples of soil. Where large quantities of soil were heated, they were usually spread out in the ovens in a layer about one inch thick. This was also true when heating was done in the autoclave for short periods of time. The temperatures were taken either by means of a mercury or electrical thermometer for low temperatures, or with a Pyrometer for temperatures above 250°C. In the later experiments, a mercury thermometer was also used for temperatures up to 500°C. It was deemed important to know approximately the rate of rise of the temperature of the soils in the autoclave. An arrangement was made by means of a packing valve and electrical resistance thermometers by which this could be done. The rise in temperature of substances placed in the autoclave was relatively rapid at first but reached the maximum slowly. According to these preliminary tests, 35 to 60 minutes, depending on the container used, should be sufficient to thoroughly heat the soil throughout in the autoclave to a temperature of 115°C. at 15 to 20 pounds of pressure in bulks as large as 2 to 3 kgm. One hour and thirty minutes was, however, always allowed for sterilization in the autoclave except where otherwise stated.

In the germination tests the soils were heated in the Petri dishes used in the tests at the lower temperatures, but in clay dishes at the higher temperatures. Fifty grams of air-dry soil was weighed out and used in all cases in the germination tests. As soon as cool after heating, water was added to the soils bringing them all as near as possible up to the same percentage of moisture, which was practically but not quite up to saturation. As soon as all the soils were evenly moistened, 100 seeds, which had previously been counted out, were placed in each Petri dish, merely being scattered over the surface of the soil. The tests were all run in duplicate. The Petri dishes retained a fairly constant supply of moisture from 10 to 12 days, and it was rarely found necessary to add more water to complete the tests. The seeds were picked out with tweezers and the number that germinated at certain intervals, usually of 12, 18, or 24 hours, were recorded. Checks on unheated soil or filter paper were always used.

In the case of heating large quantities of soil with dry heat for plant growth studies, more difficulty was encountered in getting uniformity of heating. In some of the earlier tests the soil was heated directly in the pots used, but later it was found more satisfactory to heat the soil in shallow pans which would

permit of one or two stirrings during the heating process as well as a thorough mixing afterward. After being thoroughly cooled, the soils were watered and sown to seed or seedlings transplanted on the same day or the day following heating. No special attempt was made in the ordinary tests to prevent reinfestation of the pots with organisms.

Extraction of the soil with water was made by allowing equal weights of water and soil to remain in contact for 24 hours with frequent shaking or stirring. The extract was then filtered off through filter paper, usually with reduced pressure when filtering was slow. These extracts were used for germination tests by saturating three or four layers of filter paper in Petri dishes with the solution, using water on filter paper as checks. Extracts obtained in this way were also used for the freezing-point determinations and in the temperature studies with Dewar flasks.

Freezing-point determinations were made with a Beckmann thermometer in the ordinary way, the method differing, however, from that described by Boyoucos (6), in that extracts were used instead of the soil itself. The readings are not intended to represent the actual concentration of the soil solution in the soil as they are no doubt too low, but they do give a fairly good measure of the comparative concentrations of the soil solutions used.

Ammonia determinations were made by the ordinary magnesium oxide distillation method, which again is to be regarded as giving comparative rather than actual amounts of ammonia present.

Seed germination on heated soils

The earlier workers on heated soils did not apparently note any particular effect of heated soil on seed germination. This is to be expected since it is usually only in comparative germination tests that this fact becomes most evident. Stone and Smith (71) were probably the first to carry on experiments along this line. They found an apparent acceleration of germination in heated soils and noted further that different kinds of seeds behaved differently in this respect. Pickering (50) started a fairly extensive study of the subject of the toxic action of heated soils as measured by seed germination. Unfortunately his first papers, especially, are marked by considerable lack of uniformity in results and although most of the general conclusions drawn are quite correct in principle, the data are in many cases meager and confusing. He found, for instance, in one case that heating soil to 100°C. prevented seed germination altogether, while heating to 250°C. had little effect. In reality the opposite is more likely to be the case. On the whole, however, he concludes that the time of incubation increases considerably as the temperature to which the soil is heated is increased, whereas the percentage of seeds germinating decreases. In some cases acceleration was noted on soils heated to 60° or 80°C. Heating to 200° produced the maximum retarding effect. Seeds varied in their response, mustard for instance being more affected by heating than rye. The length of time of heating soil at a certain temperature

had no special effect, but increased moisture content on heating increased the toxicity. Pickering also noted that the toxic property was retained in storage of heated soils, and that its retention was influenced by moisture content and temperature during storage.

In a second paper Pickering (51) reports that the formation of the inhibitory substance to seed germination begins at temperatures as low as 30°C. and reaches its maximum at 250°. He found most soils in their natural state less favorable for germination than water alone, and that sterilization by antiseptics is equivalent to heating to 60° to 75°C. as far as seed germination is concerned. The "poverty" or "richness" of a soil did not seem to bear any relation to its behavior when heated. Takoma soil (obtained from Whitney, U. S. Dept. Agr.) was found to be very toxic when heated and to turn seeds sown upon it black in color. Pickering attempted to show in these and later papers that the toxic property of heated soils was connected with increase in soluble organic matter, but without good success, as the exceptions found were too numerous. His results on the loss of toxicity in heated soils are especially interesting and lead him to conclude that the disappearance of the toxin is due to an oxidation process. Pickering's conclusions on the nature of the toxic substance will be discussed more in detail in a later paragraph.

Russell and his collaborators (62–64) used seed germination tests to a considerable extent as a measure of the changes induced in soils by heating and by treatment with volatile antiseptics. These results are hardly to be compared with that on soils heated to high temperatures such as those used by Pickering (50) and in the investigations reported in this paper. Russell and Petherbridge (64) do not agree, however, with Pickering in the supposition that the toxic agent necessarily disappears in storage or that it is organic in nature.

In making seed germination studies on heated soils, it at once becomes evident that a number of primary factors are concerned, together with a considerable number of secondary factors, which may influence the results secured in such a way as to obscure or exaggerate their importance. The primary factors are especially those of temperature to which the soil is heated, type of soil used, and kind of seed used in the germination test. These factors are all important and fundamental in comparing results. As secondary factors which may be considered as influencing or explaining the results secured, the following have especially been considered: length of time of heating, length of time between heating and the germination test, manner of storage following heating, percentage of moisture at time of heating, method of heating (dry or moist heat), aeration during and after heating, size of soil particles, natural toxicity of the soil, temperature of germination, aeration during germination test, percentage moisture in soil during germination, action of light during germination, size and viability of seeds, and time required for normal germination. Although all these factors could not be studied in detail, they have been given sufficient consideration to indicate the part which they might play in the results secured if they occurred as unavoidable variable factors.

Temperature of heating. The temperature to which a given soil is heated has been found to be probably the most important factor in seed germination tests on heated soils. This is well illustrated in table 2 which shows the relative rate of germination of lettuce seed in Waukesha silt loam soil heated to temperatures ranging from 50° to 350°C. It may be observed that the toxic property of the heated soil begins at 100°C., increases rapidly up to 250°C., and finally decreases at higher temperatures, having practically been lost on soil heated to 350°C. Minor variations sometimes occur which are not shown in this table, i.e., the increased germination at the lower temperatures of heating, (table 6), which condition occurs in certain soils before the temperature of retardation occurs. This is not to be taken, however, as the result of a different type of product forming in the soils heated to the lower temperatures from that produced at a somewhat higher temperature where retardation begins. It is rather to be considered as the stimulating action of a small quantity of the toxic substance produced. It may be, of course, assumed here that the low temperatures destroy the naturally present toxic property of some soils, but reasons for not assuming this to be so will appear later, although most soils do possess a natural element slightly toxic to seed germination. It will also be noted in table 2 that the toxic action is exhibited in this case quite largely by the retarding influence on germination, the final or total germination at the maximum temperature of retardation being as great as that on the not-heated soil. This is not, however, always found to be the case. More frequently, in fact, the total germination at the maximum of retardation is considerably lower than that of the unheated soils, indicating that some of the seeds have not been able to recover from the toxic agent and are in many instances actually killed.

A very considerable number of the tables presented here, as well as a number which are not included, repeatedly show the same general relation of temperatures of heating the soil and the effect on seed germination as stated above. The variation for different soils and different seeds is very great as will be shown by the following considerations. Although the temperature of 250°C. has not been determined with any fine detail as the exact turning point for all soils, its almost invariable occurrence on heating to this temperature as nearly as possible in gas and electric ovens points quite conclusively to the critical temperature for seed germination as lying close to 250°C.

Soil type. The amount of the toxic action produced on heating is very largly dependent upon the soil type used. To illustrate this point, the results shown in table 3 are presented. Seven different soils were used, all heated to 250°C. and their action on germination of lettuce seed compared with the action on unheated checks. A retardation of the germination occurred with all the heated soils used as compared with the checks. The extent of this retardation and the rate of recovery from it is, however, quite different, being greatest and slowest on the Waukesha silt loam and least and most rapid on the fine sandy loam. Although heating to a different temperature, or

using another kind of seed, might have led to results more complicated and more difficult to explain, it is worth while noting here that the extent of the toxic action does not seem to be correlated with any one common characteris-

TABLE 2

Relative rate of germination on soil heated to different temperatures; Waukesha silt loam; lettuce seed

TREATMENT	GERMINATION AFTER						TOTAL
	24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	57	89	92				92
Heated to 50°C.	73	91	94				95
Heated to 100°C.	2	50	80	84	87	90	95
Heated to 150°C.	0	25	71	80	82	85	89
Heated to 200°C.	0	6	32	49	57	67	82
Heated to 250°C.	0	0	3	15	27	35	92
Heated to 300°C.	0	77	90				95
Heated to 350°C.	41	92	95				95

TABLE 3

Relative rate of germination of seed on different soils heated to 250°C.; lettuce seed

SOIL	TREATMENT	GERMINATION AFTER						TOTAL
		24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Peat	Check	68	85	87	89			89
	Heated	0	0	1	5	16	62	84
Muck	Check	66	83	85	87			87
	Heated	0	0	0	12	69	83	90
Waukesha silt loam	Check	73	88	89	90			91
	Heated	0	2	3	7	15	30	67
Virgin sandy loam	Check	78	94	95				95
	Heated	0	0	1	36	62	72	85
Fine sandy loam	Check	75	87	88				88
	Heated	0	1	7	80	95	96	98
Clay	Check	70	81	83	86	87		87
	Heated	0	4	7	45	60	66	84
Norfolk sand	Check	77	87	88				88
	Heated	0	4	8	46	69	82	92

tic of the soils used. Statements have been made, and also contradicted, in the literature that the amount of the toxic action is in proportion to the amount of vegetable matter or humus present in the soil, or that it is corre-

lated with soil fertility. That the amount of organic matter in a soil plays a large part, and that the toxic property is formed from organic matter in one form or another is seemingly quite evident. In an attempt to illustrate this, the results shown in table 4 were secured where different amounts of quartz

TABLE 4

Influence of content of vegetable matter on the injurious action to seed germination on heating to 250°C.; cabbage seed

PERCENTAGE VEGETABLE MATTER	TREATMENT	GERMINATION AFTER						TOTAL
		24 hours	42 hours	66 hours	90 hours	114 hours	138 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ground quartz	None	10	58	72	75			75
	Heated	28	60	68	75			75
Quartz with 10 per cent peat	None	35	62	77	81			81
	Heated	4	19	68	79			80
Quartz with 20 per cent peat	None	40	74	79	81	82		82
	Heated	0	3	7	30	53	54	54
Quartz with 50 per cent peat	None	34	63	68	73	76		76
	Heated	0	0	0	10	32	47	47
Peat	None	34	70	77	79	80		80
	Heated	0	0	0	0	1	2	2
Quartz with 20 per cent manure	None	30	62	66	69	70		70
	Heated	1	21	50	64	71		71

TABLE 5

Loss in weight of soils on heating to 250°C. and on ignition

SOIL	LOSS ON HEATING TO 250°C.	LOSS ON IGNITION	ORGANIC MATTER LOST ON HEATING TO 250°C.
	per cent	*per cent*	*per cent*
Peat	5.4	68.1	7.8
Muck	1.4	14.8	9.4
Waukesha silt loam	0.83	5.4	15.3
Virgin sandy loam	0.76	5.6	13.5
Fine sandy loam	0.55	4.3	12.7
Red clay	0.43	5.5	7.7
Norfolk sand	0.35	1.9	18.4

sand and well decomposed peat were mixed and heated, and the toxicity was found to be in a large measure proportional to the amount of organic matter present. This, however, cannot be considered to approach conditions present in normal soils with varying percentages of organic matter. Table 5 shows the percentage loss of organic matter in soils heated to 250°C. as compared

with the total organic matter present as measured by the loss on ignition together with the per cent of the organic matter lost on heating to 250°C. Although the data at hand upon this subject are not sufficient to be significant, it is worth noting that the percentage of matter lost on ignition for the most toxic and the least toxic soil are too close to warrant any degree of importance being attached to them. It appears from these results, however, that peat and muck though highest in organic matter and losing the greatest weight on heating to 250°C. did not lose as large a percentage of their organic matter content as the others and were not as toxic as Waukesha silt loam which had considerable less organic matter but lost proportionately a larger amount on heating to 250°C. The indications are, therefore, that the amount of toxicity produced on heating is not proportional to its organic matter, but that it is rather proportional to the state of the organic matter or to the percentage which is actually decomposed but not taken care of by the soil itself through its powers of absorption. These probabilities will be considered more in detail under a study of the nature of the toxic substance.

That results may be quite different when lower temperatures and different seeds are used, may be seen by referring to table 6. Heating to 50°C. stimulated cabbage seed germinations on most soils. Heating to 100°C. sometimes stimulated and at other times retarded germination. Heating to 250°C. retarded germination in all cases; while heating to 500°C. brought the soil back practically to normal in all cases as far as toxicity is concerned. Heating to 800°C. either stimulated germination, or heating to such a high temperature may be regarded as having merely eliminated the inhibiting elements naturally present in soils. The difference in results at the lower temperatures from that at higher temperatures has already been explained as being believed to be due to the stimulating action of a small amount of the toxic substance. This stimulating action may be considered to be exaggerated in table 6 on account of the use of cabbage seed in the germination test which is more "resistant" to the toxic substance than lettuce seed, as will be shown in the following paragraphs. If wheat seed had been used the acceleration would have been more marked. The Waukesha silt loam soil being found to be most toxic to seed germination when heated, has been used most in the toxicity experiments.

Influence of type of seed used on results secured. The great variation in susceptibility of seeds of different species of plants to the toxic action of heated soils opens two lines of interesting inquiry: the first being as to the nature of the toxic substance produced when the soil is heated, and the other being the causes of these differences in "susceptibility." The former is the one of most concern in the problems undertaken. The latter is a physiological problem often encountered in dealing with the relation of various chemicals to plant tissues, and may have an important bearing in explaining the nature of the toxic agents in heated soils. If a correlation could be established between variations in "susceptibility" of different seeds to heated soils with that of the

TABLE 6

Effect of heating various soils to different temperatures on germination of cabbage seed

SOIL	HEATED TO	GERMINATION AFTER					Total
		48 hours	72 hours	96 hours	120 hours	144 hours	
	°C.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Peat*	Check	71	79	84			84
	50	76	80	82	83		84
	100	50	80	85	88		89
	250	21	32	40	53	65	77
	500	85	93	95			96
	800	83	86	89	93		93
Sparta sand	Check	77	87	90	92	93	93
	50	92	94	94	95		95
	100	81	90	91	92	93	93
	250	40	60	66	68	70	80
	500	73	81	86	90		90
	800	86	91	91	93		94
Red clay	Check	80	88	90			90
	50	73	78	81	82		83
	100	79	89	89	91	92	92
	250	30	47	54	57	67	92
	500	79	84	86	88	89	89
	800	79	85	87	88		91
Waukesha silt loam	Check	72	87	89			90
	50	74	84	88			88
	100	7	17	24	31	32	83
	250	4	7	8	11	13	81
	500	72	79	84	85		86
	800	79	83	87	90		90
Fine sandy loam	Check	71	80	80			80
	50	89	92	95			97
	100	91	96	98	99		99
	250	63	72	81	83		86
	500	84	87	89	90		92
	800	79	85	88	90		90
Miami silt loam	Check	77	87	89	91		91
	50	86	89	93	94		94
	100	63	86	92	93	94	96
	250	47	68	77	83	86	95
	500	71	78	80	81	84	85
	800	83	87	90	90	93	93

*An undecomposed peat, not the same as described in table 1.

toxic action of certain pure chemicals on the assumption that seeds react differently to various chemicals, good indirect evidence of the nature of the toxic substance could be established.

The reaction of seeds to chemicals or to heated soils may be either in the direction of stimulated or retarded rate of germination, or in increased or decreased total percentage germination. The experiments are, however, confined to and based mostly upon the effect on rate of germination. If we select seeds of a wide range of families of cultivated plants as is shown in table 7 and compare the germination on soil heated to 250°C. with the germination on unheated soil, it will be found that on the particular soil used, the seeds of all families are retarded in germination. It can be noted, however, that the seeds differ very markedly in their resistance to the toxic property, as indecated by the relative germination at a certain period. The rapid recovery from the toxic action by some of the species and the final increased percentage of germination of certain species above that on unheated soils is notable. The seeds of wheat and cucumber are seen to belong to the very "resistant" class, whereas those of clover and lettuce are very "susceptible" to the toxic property. Various gradations between the most and least susceptible species occur, and it is reasonable to suppose that other seeds more resistant and more susceptible than those tested here exist in nature. Working with a soil becoming very toxic on heating to the temperature most productive of toxicity (i.e., 250°C.), the advantage in simplifying the facts, over that on heating this soil to a lower temperature, is to be seen in table 8, where Waukesha silt loam was heated to 115°C. and sown to different seeds. In this case, we have no retardation but stimulation of the most resistant species together with marked retardation of the "susceptible" species, and it becomes easier to misconstrue the nature of the toxic properties present.

Various factors of secondary importance influencing germination on heated soil. Turning to some of the other factors mentioned which deserve consideration as influencing or explaining the results noted above, it may be well to consider them briefly at this point, illustrating by data only those which may be especially important or interesting. In all experiments on seed germination and plant growth the seed has usually been sown as soon as possible after heating the soil, this time rarely exceeding 6 hours, and where delayed has been usually due to difficulty in wetting certain of the soils uniformly after heated to a low temperature. Naturally, however, the question arises as to what may happen in soils stored under different conditions for a considerable period of time between heating and seeding. All experiments of this nature have gone to show that the toxic property remains practically unchanged if the soil is kept in a dry condition, but that if the heated soil is kept in a moistened condition for a considerable period of time, changes occur and the toxic property is gradually lost or destroyed. This point is illustrated in table 9 where two different soils heated to 250°C. were kept for three months in the moist and dry condition, the toxicity as shown by seed germination being entirely lost in the

TABLE 7

Relative rate of germination of different seeds on soil heated to 250°C.; Waukesha silt loam

SEED	SOIL TREATMENT	GERMINATION AFTER							TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	186 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Garden cress	Check	26	34	44	62	67	68		68
	Heated	0	1	1	2	3	5	15	24*
Wheat	Check	36	76	86	93				93
	Heated	11	68	87	94	95		-	96
Lettuce	Check	69	72	73	83	85	88		88
	Heated	0	0	1	2	4	7	27	50*
Clover	Check	44	68	79	85	86			86
	Heated	0	3	3	5	7	9	37	68*
Flax	Check	96	97	98					98
	Heated	21	57	71	86	90	92	95	95
Cucumber	Check	41	65	75	84	85	86	87	88
	Heated	23	54	68	82	84	85	87	88
Cabbage	Check	33	53	59	65	65	66		66
	Heated	0	0	1	3	11	29	51	56*
Onion	Check	3	16	45	73	78	78	80	80
	Heated	0	1	8	54	70	73	75	75
Spinach	Check		5	15	37	48	51	57	59*
	Heated		0	1	5	13	20	36	47*
Buckwheat	Check			8	20	29	46	61	63
	Heated			1	12	22	37	60	64
Carrot	Check			43	59	64	67		67
	Heated			0	2	15	42	63	68
Tomato	Check				30	54	58	65	67
	Heated				0	1	10	53	61
Phlox	Check				7	11	13	20	24
	Heated				0	0	0	3	11*
Snapdragon	Check				6	31	41	47	49
	Heated				0	0	0	1	8*
Cotton	Check				5	14	39	72	74
	Heated				0	1	11	65	73
Linaria	Check					17	39	58	67*
	Heated					0	0	0	2*
Parsnip	Check						4	12	17*
	Heated						0	4	8*

* Seeds moldy and failure to show a greater total germination may be partially due to this.

moist state but quite largely maintained in the dry state. This persistence of the toxic agent is further shown in table 10 where heated soil kept dry for two years still showed a very considerable toxic action. That aeration has no particular influence in this regard where soil is stored dry but that it has

TABLE 8

Relative rate of germination of different seeds on heated soil. Waukesha silt loam heated to 115°C.

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	258 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Clover	None	65	81	85	86	88		88
	Heated	57	74	80	84	87	88	88
Cabbage	None	56	83	88	90	91	92	92
	Heated	69	82	87	88	89	90	91
Garden cress	None	20	48	64	68	71	72	73
	Heated	16	40	52	56	60	66	66
Onion	None	2	67	93	95	96	97	97
	Heated	4	64	93	96	97		97
Buckwheat	None		7	14	23	33	55	55
	Heated		3	14	21	29	42	42
Tomato	None		9	84	96	97	98	98
	Heated		2	69	91	98	99	99
Carrot	None		10	36	53	58	64	64
	Heated		3	24	50	62	67	67
Lettuce	None	83	92	94				94
	Heated	8	40	67	83			83
Flax	None	97	98					98
	Heated	92	94	95				95
Wheat	None	30	93	97				97
	Heated	47	96	97				97
Cucumber	None	5	65	82	84	85		86
	Heated	10	71	86	87	88		88

some influence where soil is stored moist, is shown in table 11, in which experiment, soils were stored in Mason jars with covers sealed on in one case, but left off in others. No consideration was given to reinfestation with microorganisms in this experiment. This test indicates that the time of seeding following sterilization or heating has little influence if the soil is kept

TABLE 9

Effect of storage of heated soil for 3 months in moist and dry condition upon rate of seed germination; cabbage seed

SOIL	TREATMENT	STORED	GERMINATION AFTER					TOTAL
			43 hours	55 hours	67 hours	79 hours	97 hours	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam	Check	Dry	49	53	70	73	73	75
		Moist	53	65	70	74	76	78
	Heated to 250°C.	Dry	13	25	38	50	60	72
		Moist	50	61	66	71	73	75
Fine sandy loam	Check	Dry	43	53	62	66	66	66
		Moist	45	55	62	64	66	68
	Heated to 250°C.	Dry	19	39	50	61	64	70
		Moist	49	65	68	73	76	78

TABLE 10

Influence of two years storage of dry heated soil on relative rate of seed germination; Waukesha silt loam; clover seed

SOIL TREATMENT	STORED DRY	GERMINATION AFTER (PERIOD)						TOTAL
		1	2	3	4	5	6	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	0	41	80	86				86
Heated to 250°C.	0	0	2	4	7	11	14	53
Not heated	2 years	20	75	81	83	85	87	87
Heated to 250°C.	2 years	0	11	22	42	66	70	75

TABLE 11

Influence of moisture and aeration of heated soil during storage on germination of seed; Waukesha silt loam; lettuce seed

TREATMENT	STORAGE		GERMINATION AFTER						TOTAL
	Water	Air	24 hours	36 hours	48 hours	60 hours	72 hours	84 hours	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	+	+	75	95	96				96
		−	26	87	91	92			92
	−	+	3	62	89	92			92
		−	11	76	87	91			91
Heated to 250°C.	+	+	0	10	58	73	74	74	
		−	0	2	19	48	51	52	
	−	+	0	1	1	2	8	9	
		−	0	0	0	2	4	5	

dry, but that the toxicity is gradually lost where heated soils are kept moist and exposed to aeration and contamination. It points further to the fact that some of the toxicity to seed germination may be diminished toward the close of a seed germination test in Petri dishes, and also that a part of this, which if given off from the soil rather than destroyed in it, may be partially retained in the enclosed chamber due to lack of good aeration, and hence exaggerate the retardation which fact may partly explain the more marked retardation under these conditions than is ordinarily observed on heated soils under natural conditions.

That the amount of moisture present in the soil at the time of heating may influence the amount of toxic substance formed or its retention by the soil is shown in table 12. Here 50 gm. of Waukesha silt loam were placed in Petri dishes and varying amounts of water added before heating in the autoclave, after which it was made up to equal percentages of water and seeded to lettuce.

TABLE 12

Relative rate of germination of seed on soil heated with different amounts of moisture; Waukesha silt loam; lettuce seed

TREATMENT	WATER ADDED TO 50 GMS. AIR-DRY SOIL	GERMINATION AFTER					* TOTAL
		30 hours	42 hours	54 hours	66 hours	78 hours	
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated		76	89	91	94	95	96
Heated 115°C.	0	11	29	68	87	89	93
	5	17	49	79	89	92	92
	10	10	27	71	87	91	92
	15	6	23	63	82	85	89
	20	4	19	61	80	87	94
	25*	2	16	52	80	85	90

* Saturated.

Increased water content at the time of sterilization apparently increases the toxic action. There is also, however, an indication that adding a small percentage of water may be less productive of injurious products than heating an air dry soil, although this may be associated with physical changes, since the ability of relatively dry soil heated to 115°C. or thereabouts, to absorb moisture is very poor. Throughout the experiments, the soils used for heating have been usually air dried, this of course, being advantageous in heating to higher temperatures with dry heat.

As to time of heating, an arbitrary standard must be chosen and maintained as closely as possible, the soil being allowed to become gradually and as uniformly heated to the desired temperature as possible within that period of time. Where large quantities of soil are heated it is important to mix the heated soil thoroughly before taking fractions for tests. To determine the effect of length of time of heating a soil on seed germination, the Waukesha silt loam was heated for different lengths of time, ranging from 10 to 160 minutes, in the

autoclave at 115°C. (table 29). The soils were spread out in a thin layer to make thorough heating at the short periods of time possible. Ammonia determinations and freezing point determinations of the soil solution were also made in this experiment. The differences due to length of time of heating at this temperature were small and perhaps insignificant, but there is seemingly a slight increase on increased time of heating followed by a lowering of toxicity to seed germination which then remains fairly constant and although changes do occur they do not seem to be constantly in one direction. This was seemingly not correlated with ammonia content or concentration of the soil solution. Similar rise and fall of toxicity was also obtained for soil heated to 200°C. for 2, 4, 6, 8, 10, and 12 hours, but no decisive conclusion could be drawn as to the influence of time of heating on seed germination.

TABLE 13

Effect of temperature of germination on toxic action of heated soil; Waukesha silt loam; red clover

SOIL TREATMENT	TEMPERATURE OF GERMINATION	GERMINATION AFTER					TOTAL
		48 hours	72 hours	96 hours	120 hours	144 hours	
	°C.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	12	5	40	68	80	81	82
	15	14	52	67	73	76	79
	21	39	79	90	91	92	92
	24	77	88	91	91	91	91
	26	75	86	86	87	87	87
	35	27	37	44	56	60	
Heated to 250°C	12	0	0	2	9	19	77
	15	0	1	5	10	19	85
	21	0	5	12	20	25	83
	24	6	15	21	40	67	71
	26	2	3	5	27	58	60
	35	0	0	0	0	1	

To determine the relation of the temperature at which the germination test is made upon the degree of toxicity shown by heated soils, the results shown in table 13 were secured. Waukesha silt loam soil was used, and the heated and unheated checks were placed at different temperatures in a series of Altmann and other electrically regulated incubators where constant temperatures could be maintained. The optimum temperature for the germination of clover seed was found to be close to 24–26°C. The same temperature apparently held for germination in both heated and unheated soils. The proportion of germination seemingly holds the same for all temperatures on both heated and unheated soils excepting at the highest temperature, i.e., 35°C. where the injurious action of the combination of high temperature and soil toxicity is proportionally greater for the heated soil. It is apparent, however, that a fairly large range of temperature variations will not materi-

ally effect results in the germination experiments. No special efforts were, therefore, made to keep all germination tests at constant temperatures, and usually only room temperatures (about 20–22°C.) were used. These were kept quite constant day and night, however, by the automatic regulation of the steam-heating system.

In the preceding temperature test of germination, lettuce seed was used in the first trial. It was found that the lettuce seed germinated very poorly in all the dishes in the incubators which excluded light. It was at first thought that poor seed had been used but seed from the same samples in the laboratory germinated normally. It was, therefore, concluded that light must be a controlling factor in lettuce seed germination, and this was found to be the case. It has, of course, been repeatedly shown that light is necessary for normal germination in many seeds (38). As lettuce seed was most commonly

TABLE 14

Influence of absence of light on the germination of lettuce seed in heated and unheated soil; Waukesha silt loam

SOIL TREATMENT	LIGHT	GERMINATION AFTER					TOTAL
		30 hours	48 hours	72 hours	96 hours	120 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	+	75	88	90	93	95	97
	−	1	7	8	9	10	10
Heated to 115°C.	+	12	40	62	74	84	90
	−	3	4	5	5	6	8
None	+	82	93	93	94	95	96
	−	0	10	16	20	21	22
Heated to 250°C.	+	0	2	8	15	23	26
	−	0	3	7	8	12	13

used in the tests in my experiments, however, on account of their susceptibility to the toxic action of heated soils and their ease and convenience in handling, it was found necessary to keep the seeds germinating in the open room rather than in a dark incubator. An interesting fact noted in this connection (table 14) was that the combined action of absence of light and of soil toxicity did not retard seed germination in proportion to the expectations when compared with unheated soils in the absence of light. In other words, the heated soils apparently supplied in some small measure a substitute for light in germination. This is especially interesting considering the results of Gassner's work (20) in replacing the effect of light with certain nitrogenous salts.

In an effort to determine the relation of size of soil particles used to germination on heated soils, a heated sample was screened to different sized

particles and sowed to lettuce. No particular conclusion is warranted from this test; although there is an indication that the larger-sized particles are less toxic than the finer grades. It is possible, however, that other factors than toxicity play a part here. The results are in agreement with those of Stone and Monahan (70) on this subject, and as suggested by them the precaution should be taken of using soils of a standard degree of fineness for germination tests. The addition of ammonia to soils of coarse and fine textures indicated more markedly that greater toxicity occurred in soils of fine than in those of coarse particles.

Pickering (50) has shown that moisture content of soil in germination tests may vary quite widely without greatly influencing the results. This is in accordance with results obtained in an experiment carried on by the writer in this connection. Too low moisture content reduces, of course, the rate of germination, while the higher moisture contents tend to increase rate of germination, but probably reduce total germination slightly. It has already been mentioned that certain soils heated to low temperatures take up water with great difficulty. The loss of capillarity is in some instances very striking, the soil seeming in fact at times to have lost all affinity for water. In "kneading" the soil up with water in order to get some degree of uniformity of moisture throughout, it is possible that a physical condition is obtained unfavorable to germination quite apart from toxic action. This influence, however, has not been deemed sufficiently important in this connection to warrant detailed study. Soils heated to high temperatures do take up water very much more readily than unheated soils, and it was found, for instance, that a virgin sandy loam heated to 250°C. took up water two to three times as fast as the unheated, while this soil heated to 115°C. took up water only one-half to one-eighth as fast as the unheated soil.

The size of the seed is not considered as influencing the susceptibility to the toxic action of the heated soil. Corn and peas, for instance, being larger than rye or buckwheat, are more susceptible, while clover and lettuce being smaller than the latter, were also more susceptible. Similarly the gross chemical composition, as for instance starchiness and oiliness, does not seem to be in any way connected with susceptibility or resistance to injury. Sweet corn, for example, is only slightly more susceptible than field corn, and flax and wheat are about equally resistant. Age, or maturity of the seed, as far as noted does not apparently influence results to any extent. It seems most probable that the factor which determines the nature of the response in seed germination lies in the selective permeability of the seed coats to certain chemical substances. With the above facts in mind tests were begun with the idea of determining and measuring more closely the nature of the toxic substances in heated soils. Before taking this up it is probably advisable to consider how far these facts concerning seed germination are correlated with plant growth on heated soils.

Plant growth on heated soils

Although the beneficial action of heated soils on the growth of the higher plants had been known for a long time, it was apparently not until about the middle of the last century that any serious experimental investigation was undertaken in this field. Struckman (73) in 1857 in addition to other lines of work on burned soils obtained good results with cabbage and rape, but noted no especially good results with vetch and flax on burned soil. The experiments of Fleischer (17) and his associates at the "Moor-Versuchs Stationen" were continued for a number of years, heated soils giving on the whole good results with plant growth, although the economy of burning was open to question. With respect to the influence of dry heat on soil, the more recent investigations along this line are especially those of Pickering (53), Seaver and Clark (68), Fletcher (18), and Kelley and McGeorge (30). A more extensive amount of literature exists upon plant growth in steamed soils, and there is a great deal of evidence showing the beneficial action of steamed soils on plant growth since the time of Franke (19), Pfeiffer and Franke (49), and Kruger and Schneidewind (37). Studies on the injurious action of heated soils began especially with the results of Deitrich (13), and these have since become a very important part of the investigations upon the subject. Following the work of Girard (21) who applied carbon bisulfide to the soil used for the growth of several agricultural crops, a large amount of literature has accumulated with the object of explaining the beneficial action on plant growth of antiseptics applied to soils. That sterilization by chemical agents and by heat have one and the same effect on soil as far as plant growth is concerned has been generally accepted, and the two methods, sterilization, or "partial sterilization" by heat or chemicals are, therefore, from a biological standpoint, usually considered together by most authors. The theories concerning the causes of the modification of plant growth on sterilized soils have already been briefly mentioned, and the changes in and caused by sterilized soils outlined. The experimental results presented here are mainly concerned with establishing further evidence relative to the toxic and beneficial action of heated soils, and the immediate and subsequent changes produced on different soils as measured by the growth of different plants, with the purpose of arriving at a better conception of the nature of the beneficial and toxic substances produced in heated soils. Experiments were therefore undertaken to determine the influence of the following as measured by plant growth: (a) the response of different types of soil to sterilization; (b) the effect of different temperatures of heating; (c) the effect on different kinds of plants; (d) the influence of different lengths of time of heating; (e) the influence of repeated heating; (f) the influence of various soil environmental factors on the expression of the injurious and beneficial action.

Soil type. The remarkable variation in results which may be secured with plant growth on different soils when sterilized is best illustrated by table 15. As wide a variety of soil substances as could be secured were used including those previously described, and also pure leaf mold, greenhouse compost, and a very acid sand (lime requirement 9.38 tons per acre). After being sterilized at about 110°C. these soils together with their checks were planted to tomatoes. A wide variation in results was noted from the start, both in quantitative and "qualitative" response, and these variations and changes continued to occur throughout the period the plants were allowed to grow. Comparing the yield of the different unsterilized soils it may be noted that the compost was apparently the most fertile and the red clay the least fertile for tomatoes. The greatest percentage increase in yield, however, occurred on the muck soil 275 per cent and the greatest reduced yield on the virgin sandy loam where the plants were practically all killed (plate 1, fig. 1). There was manifestly no correlation between the fertility of the soil and the toxic or beneficial action. Considering total organic matter, in those soils in which the loss by ignition has been determined (table 1) it may be seen that peat, highest in organic matter (68.1 per cent), though very toxic on being sterilized, was not so much so as virgin sandy loam with only approximately 5.6 per cent organic matter. Muck with about 14.8 per cent organic matter was only slightly more beneficially influenced than Miami silt loam with only about 5.4 per cent organic matter. There is, therefore, also apparently a lack of correlation between the behaviour of the heated soils toward plant growth and their content of organic matter. That fertility and organic matter bear a close relation to the action on plant growth upon sterilizing the soil, has been repeatedly suggested by various writers but frequently disproven when subjected to experimental test. That the organic matter should bear some relation to the toxic and beneficial action upon heated soils is, however, too evident in connection with other facts to dismiss the matter without further consideration. The influence of the organic matter may be indirect and complicated by various factors, especially by its relation to the absorptive capacity of the soil, its chemical state as influencing extent of decomposition upon heating, and by its relation to bacterial activity subsequent to sterilization. The relation of absorptive capacity, especially, will be considered in connection with the discussion of the nature of the toxic agent. For the present we can only conclude that very little is suggested by the gross character of the soil itself as to its action upon plant growth when subjected to sterilization by heat.

Aside from simple retardation and acceleration of growth, several other interesting results were obtained where tomatoes were grown in different soils heated to about 110°C. These are indicated briefly in table 15 but will be discussed in more detail under the subject of the "qualitative" action of heated soils. The extreme toxicity of virgin sandy loam to tomatoes when sterilized should not, however, be allowed to confuse the problem being pri-

TABLE 15

Air-dry weight of tomato vines grown on different soils sterilized at 110°C.*

SOIL	TREATMENT	DRY WEIGHT	PER CENT INCREASE (+) DECREASE (−)	EARLY GROWTH	LATE GROWTH
		gm.			
Miami silt loam	None	1.60			Leaves yellowing
	Sterilized	5.65	+254	Leaves mottled; slight retardation	Leaves a healthy green
Muck	None	1.60			Leaves yellowing
	Sterilized	6.00	+275	No retardation	Leaves a healthy green
Fine sandy loam	None	1.45			
	Sterilized	3.05	+110	Slight retardation	
Norfolk sand	None	0.50			
	Sterilized	0.90	+ 80	Purple pigmentation	
Leaf mold	None	6.20		Purple pigmentation	
	Sterilized	6.95	+ 12	Leaves mottled; slight retardation	
Red clay	None	0.15		Purple pigmentation	
	Sterilized	0.15	0	Purple pigmentation; no retardation	
Peat	None	0.90			
	Sterilized	0.30	−200	Marked retardation; purple pigmentation	Change in pigmentation and recovery started
Waukesha silt loam	None	4.60			
	Sterilized	2.10	−119	Uneven retardation	Marked toxic action on some
Acid sand	None	0.85		Purple pigmentation	
	Sterilized	0.35	−142	Purple pigmentation; slight retardation	Several spots and lesions on leaves and stems
Greenhouse compost	None	9.65			
	Sterilized	5.35	− 80	Leaves mottled; slight retardation	Toxic action started on leaves
Virgin sandy loam	None	5.60			
	Sterilized	0.15		Marked retardation; lesions on leaves	Plants practically killed by lesions on leaves and stems

* Six plants—62 days' growth in duplicate pots.

marily considered, i.e., simple retardation. This extreme toxic action may not be due to the same cause as that of simple retardation. The percentage decrease on the virgin sandy loam is not included in the data given. This type of injury in which plant tissue is actually destroyed, has been termed "chemical" injury in order to distinguish it from retardation where no such injury appears to occur. That "chemical" injury is not confined to virgin sandy loam soil alone, however, is evidenced by the same but less marked occurrences in three of the other soils used. The results secured in table 15 would have been quite different if other plants had been used, as will be shown later. On the other hand, tomatoes are not the only plants subject to forms of "chemical" injury. Furthermore the temperature to which the soil is heated, the length of time of heating, and the temperature of the soil during the growing period are all influential in determining the occurrence and extent of injury resulting. The results presented in table 15 only show, therefore, what may occur under a certain set of conditions when various types of soil are used. It should also be noted that the results as shown by the final yields are only comparative for that stage of plant growth at which the crops were harvested. It requires a longer period for some soils or plants to recover from the toxic action than others, and in certain instances the greater the toxic action and the time required for recovery, the more pronounced the subsequent increase. The yields should preferably be taken therefore at the maturity of the plants. In experiments under greenhouse conditions this is frequently impossible with certain crops and is not regarded as essential with others. The yields given are therefore in terms of dry vegetative matter produced during a certain period of time regardless of maturity but usually at a stage when conditions similar to that which may be expected at maturity have been reached. Percentages of increase or decrease in yields, therefore, may not indicate the maximum of either but rather the comparative yields at a certain time. On the other hand it is possible so to regulate the time of taking the yield, by noting the stage and vigor of recent growth, that any serious error in this respect may be avoided.

In connection with the studies on influence of soil type, an experiment was conducted to determine whether any difference existed between surface soil and subsoil in their behavior toward sterilization. Stone and Monahan (70) convey the impression that such a difference exists owing to the location of the soils rather than to their chemical or physical characteristics, although it is probable that the authors did not intend to convey such an impression. These writers report a decrease in yield of soy beans in sterilized subsoil as compared with sterilized surface soil. These results could not be corroborated. With soy beans a Miami silt loam surface soil showed an increase of 91.3 per cent and the subsoil an increase of 118.7 per cent over the unsterilized soil. Virgin sandy loam surface soil showed a decrease of 20 per cent for the surface soil and an increase of 58.3 per cent for the subsoil. These tests

though not carried out in detail, indicate that nothing definite can be concluded from the location of the soil with reference to the surface as to its action to plant growth upon sterilization. The results are, on the other hand, not correlated with content of organic matter in the soil as suggested by Stone and Monahan's conclusions.

Temperature of heating. The temperature to which a soil is heated has a marked influence upon its behavior toward plant growth as is illustrated by a preliminary experiment (table 16) where cabbage was used on two different soils heated to 115°, 250°, and 350°C. The early plant growth was retarded to the greatest extent on both soils on heating to 250°C., but the subsequent beneficial action is seen to be the greatest at this temperature of heating. The muck soil gave a decidedly greater beneficial action on heating than did the Waukesha silt loam, but it is not to be expected that all soils will show beneficial action at a given time after heating to 250°C., nor that all plants will

TABLE 16

Dry weight of cabbage produced in soil on heating to different temperatures

TEMPERATURE OF HEATING	MUCK SOIL		WAUKESHA SILT LOAM	
	Weight	Increase	Weight	Increase.
°C.	*gm.*	*per cent*	*gm.*	*per cent*
Check	1.95		2.55	
115	8.60	340.7	3.40	33.3
250	9.70	402.5	4.95	94.1
350	4.35	123.0	3.60	41.1

respond in the same manner. On heating six different soils to 250°C. the increase in yield of tobacco, for example, ranged as follows: Muck, 571 per cent; Waukesha silt loam, 473 per cent; clay, 150 per cent; fine sandy loam, 96 per cent; virgin sandy loam, 62 per cent, while the peat soil showed a decrease of 25 per cent. The retarding influence of the peat, however, would have been represented by a much greater figure earlier in the test, while, on the other hand, at the time of cutting the plants they were growing vigorously, indicating that in two or three weeks the yield on the heated peat would have exceeded that on most of the other heated soils. Such seeming discrepancies in results do not alter the fact, however, that there exists a maximum degree of toxicity followed usually by a maximum degree of beneficial action to plant growth on soils heated to approximately 250°C. That this critical temperature lies closer to 250°C. than to 200° or 300°C. has been repeatedly noted and is illustrated in table 17 where the relative growth at the end of one month (placing the largest growth at 100), together with the final yield is shown. In a similar manner the retarding action of virgin sandy loam to tomatoes is shown in plate 1, figure 2 and the benefits of heated muck soil on radish is shown in plate 1, figure 3. The decreased yield (table 17)

on heating to 50°C. is believed to be due to loss of nitrates or other forms of nitrogen on drying, together with no beneficial action in the way of increased availability of plant food, due to heating at this low temperature. While tobacco recovered relatively rapidly from the toxic action, other plants may require a relatively much longer time (plate 2, fig. 1), and may even in some instances entirely fail to recover during the normal life of one annual plant, although such "recovery" of the soil may be shown in the later crops. The time required for recovery is, usually, in proportion to the degree of toxicity; consequently we find that on soils heated to different temperatures the earliest growth is usually best on those soils not heated or on soils heated to 350°C. or above, where no toxic principle is present. If the toxicity at 100°C. is relatively small a rapid recovery occurs and in the space of two weeks to a month it will usually be found that the plants on soils heated to this temperature are most vigorous owing to the beneficial action obtained.

TABLE 17

Influence of heating soil to different temperatures on yield of tobacco; Waukesha silt loam

TEMPERATURE OF HEATING	RELATIVE GROWTH AT END OF MONTH (ESTIMATED)	DRY WEIGHT	INCREASE
°C.		gm.	per cent
Check	95	1.55	
50	60	1.15	—34
100	100	2.70	74
150	90	4.85	212
200	85	5.20	235
250	75	6.40	312
300	85	5.90	280
350	95	4.05	161

At this time, however, the plant growth at the 250°C. temperature will usually be the poorest with less retarding action at the 200° and 300° temperatures. Recovery then follows at 150°C., 300°C., 200°C., and finally at 250°C. Once the injurious action is lost, the increase in plant growth occurs relatively rapidly and at a time when the soils heated to the lower temperatures seem to have exhausted the beneficial property produced, the soils heated to 200° and 250°C., may be at their height of vigor of growth. The apparent correlation between the action of soils heated to different temperatures on plant growth and their action on seed germination is evident, and seemingly indicates that the toxic principle is the same in both cases.

Results vary with kind of plant grown. The extreme toxicity of heated virgin sandy loam soil to tomatoes has been shown. If now, different types of plants are grown in this soil, heated to the same temperatures (table 18), it is found that certain plants, in fact most plants, thrive on this heated soil which is so toxic to tomatoes as to practically kill them in many instances (plate 2, fig. 2). Buckwheat and wheat show an increased growth of 321.8

per cent and 245.9 per cent, respectively, while tomatoes show a decrease of 41.7 per cent (table 18). This experiment was conducted during the summer and the decrease is considerably less than that which may be expected to occur during cooler seasons of the year, as will be shown later. That this difference in "resistance" and "susceptibility" of plants to the toxic action of heated soils is characteristic on all soils and with all plants cannot be doubted although no attempt has been made to prove this point. The results obtained at any one time are always complicated by a considerable number of factors, of which probably the most important is environment, and consequently the results are likely to be confusing unless properly analyzed. The

TABLE 18

Effect of sterilization of soil at 115°C., in autoclave on yield of different crops; virgin sandy loam

CROP	TREATMENT	DRY WEIGHT	INCREASE	REMARKS
		gm.	*per cent*	
Buckwheat	None	3.20		
	Heated	13.50	321.8	Early growth markedly retarded
Radish	None	2.15		
	Heated	8.45	293.0	Early growth retarded
Soy beans	None	8.10		
	Heated	8.05	0	Early growth retarded. Foliage shows "chemical" injury
Wheat	None	1.85		
	Heated	6.50	245.9	Early grow retarded
Lettuce	None	0.80		
	Heated	1.60	100.0	Early growth retarded
Tomatoes	None	1.15		
	Heated	0.67	−41.7	Early growth retarded. Marked "chemical" injury

results of one other experiment may be cited, however, in this connection in which three different soils heated to 100°C. were grown to four different crops. Muck soil gave an increase of 140.9 per cent for radish as compared with 40.6 per cent for lettuce. Heated fine sandy loam increased the yield of wheat 119.1 per cent, but decreased the yield of lettuce 106.6 per cent, and virgin sandy loam increased the yield of radish 1.7 per cent as compared with an increase in this case of 76.4 per cent for tomatoes. These results illustrate further the marked variation in the response of plants to sterilized soils. Other results of this nature have shown in general that the Gramineae as a whole are relatively resistant to the toxic action and consequently show relatively high beneficial results from heated soils. The Solanaceae and Leguminoseae

are on the other hand apparently more susceptible to the toxic action, although great variation exists within the families in this respect. This seems roughly to correspond with the action on seed germination, but experiments to be referred to later along this line apparently fail to bring out any decided correlation.

Length of time of heating. The work of Seaver and Clark (68) and of Pickering (50) have indicated that the length of time the soil is held at a certain temperature does not materially influence its behavior toward plant growth. Some preliminary experiments on this aspect of the problem tended to corroborate their conclusions, but on the other hand there appear to be too many exceptions to permit of applying this conclusion as a general rule. Fine sandy loam soil heated to 115°C. for various lengths of time ranging from 10 to 180 minutes and planting to wheat showed that a gradual increase in yield occurred up to 120 minutes of heating but fell off again slightly on heating to 180 minutes. In this case it should be noted that a soil producing only a

TABLE 19

Influence of length of time of heating virgin sandy loam at 115°C. on growth of tomatoes

TIME OF HEATING	DRY WEIGHT AVERAGE TRIPLICATES
minutes	*gm.*
Check not heated	2.28
10	5.57
20	4.77
40	2.63
80	0.50
160	0.10

slight toxic action on heating and also a plant resistant to this toxic property were used. When tomatoes were grown on muck soil a different result was secured. Heating 10 minutes gave an increase in yield of 132 per cent, 20 minutes an increase of only 53 per cent which on heating 80 minutes was again raised to 157 per cent, but again fell off to 118 per cent on heating 160 minutes (plate 3, fig. 1). This type of behavior both with seed germination and plant growth has, however, been observed too frequently to dismiss as insignificant. The behavior seems to indicate that the balance between the toxic and beneficial action is altered by the length of time of heating, probably due to the altered balance between the production and the volatilization of one or more products from the soil on heating. More definite evidence has been secured, however, to show that the length of time of heating may have a marked action on the behavior toward plant growth. Virgin sandy loam was heated for various lengths of time between 10 and 160 minutes at a temperature of 115°C. and set to tomatoes (plate 3, fig. 2). The toxic action as shown by "chemical" injury first appeared in the soil heated for 160 minutes; and appeared successively in the other soils to and including 20 minutes of heating. At 10 minutes an almost immediate bene-

ficial action was found and persisted throughout the experiment. The soils heated for 20 and 40 minutes recovered in considerable measure and made a better growth than the checks, but they still showed the toxic action on the lower leaves at intervals. The soils heated for 80 and 160 minutes gave practically no growth of tomatoes, the leaves dying off almost as rapidly as formed. The average yield from triplicate pots are shown in table 19. It is at least very evident that the length of time of heating certain soils does influence the growth of certain plants.

Repeated heating of soils. Practical plant culturists using sterilization by heat often inquire as to whether soils once sterilized may again be sterilized and replanted with the assurance of results as good as produced by the first sterilization. Some experimental evidence has been secured on this point in two different ways: first by repeatedly sterilizing a soil after each successive crop, and second, by sterilizing a soil on successive days before planting. The results of the first tests, in which fine sandy loam was used and grown to lettuce, indicated that the soil responded in a beneficial way after each sterilization in very much the same way as previously unsterilized soils did. In another experiment in which wheat was used on muck soil and on virgin sandy loam, and the same soil was heated 1, 2, 4, and 8 times on successive days to 115°C. for 90 minutes, no marked difference in results was obtained, a beneficial action being exhibited in all cases above the unheated check. Some indication, however, that one heating was more beneficial (or less productive of toxic action) than repeated heating, is indicated by the figures (table 20). Another experiment conducted in essentially the same manner, using greenhouse compost grown to tomatoes, again indicated that repeated heating on successive days increased the toxicity to tomatoes, although all the heated soils eventually recovered and produced final yields greater than the unheated checks (table 20). It does not follow, however, that repeated sterilization of the same soil may not be disadvantageous in certain cases and for other reasons not discussed here.

Influence of temperature of soil following sterilization. The results obtained relative to the toxic or beneficial action of heated soils are found to be influenced to a considerable degree by the temperature of the moist soil following sterilization, and this may explain some of the variations which have been secured in different experiments carried on at different seasons of the year. Russell (64) and Koch and Luken (32) have previously noted that results secured on heated soils varied with the time of the year, although they did not apparently connect up this idea with soil temperature in particular. Pickering (50) in studying the loss of the toxic action of heated soils found that its temperature influenced the loss of toxicity to seed germination. It was noted by the writer, in connection with some experiments dealing with the influence of soil temperature on the development of the root-rot disease of tobacco (*Thielavia basicola*), in which steam sterilized soils were used as checks, that retarding action and chemical injury were considerably more marked at soil

temperatures below about 25°C. than at higher temperatures (plate 4, fig. 1). This toxic action was so great as to materially interfere with the progress of the experiments, and it finally became necessary to use special methods of sterilization to avoid it; although the toxic action could be materially reduced by proper methods of handling the soil after heating and before planting. In order to study this action further experiments were conducted with virgin sandy loam soil heated to approximately 100°C. and planted to tomatoes in soils kept at various temperatures together with checks in unheated soils. The tomatoes in unheated soils at soil temperatures below 20°C. made a poor growth and showed increased pigmentation, while those at soil temperatures above 23°C. and up to 32°C. made a good growth (plate 4, fig. 2). In the heated soils the tomatoes at 23–24°C. were practically killed by the toxic agent. The toxic action at lower temperatures was also great but not as

TABLE 20

Effect of repeated heating of soil on successive days to 115°C. on yield of crop grown

SOIL TREATMENT	DRY WEIGHT		
	Muck soil (wheat) average of duplicates	Virgin sandy loam (wheat), average of duplicates	Greenhouse compost (tomatoes), average of triplicates
	gm.	*gm.*	*gm.*
Not heated	4.75	4.85	3.97
Heated once	9.10	7.35	9.73
Heated twice	7.70	6.90	9.00
Heated four times	8.80	5.65	7.50
Heated five times			8.17
Heated eight times	6.55	7.00	

marked as at 23–24°C. At a temperature of 27–29°C. practically no "chemical" injury occurred, although some retardation in growth took place. It is very evident from this and other experiments that the results secured with heated soils are to a great extent influenced by the temperature of the soil. This response is believed to be due largely to differences in bacterial activity as influencing loss of toxicity of the heated soil. The reasons for this explanation will be evidenced by the experiments described later.

Other factors concerned. In addition to the factors already mentioned as being influential in determining growth on heated soils, several others would bear discussion at this time. One of the most important factors, which will be left for later discussion, is the reinfestation of the soil with normal soil flora which has a fundamental influence on the toxic and beneficial properties exhibited. Among the minor factors should be mentioned that of the moisture content of the soil both during heating and during subsequent plant growth. Excessive moisture in the soil during heating as well as during plant growth seemingly predisposes the plant to the injurious action, or increases

the amount of the toxic property in the soil. At any rate watering should be carefully regulated in heated soils, especially in experimental work. This is particularly true for the reason that once a sterilized soil is overwatered, great difficulty may develop in attempts to dry it out rapidly.

The period between the time of heating and the time of planting will influence the amount of toxic and beneficial action exhibited, if the soils are kept under ordinary conditions for plant growth during that time, but not if the soils are kept dry. Whether atmospheric conditions except in so far as they influence soil environment are of any degree of importance in determining the extent of the action of heated soils is not yet known; although they presumably may show some influence as suggested by Russell and Petherbridge (64).

Qualitative changes in plant growth on heated soils

The differences to be found in the response of seeds and plants to heated soils, aside from the more common measurable phenomena, are frequently so marked as to distinguish the heated from the unheated soils. These differences have been generally noted by practically all workers on sterilized soils in the increased vigor and deeper green color of plants grown on sterilized soils. Russell and Petherbridge (64) have gone considerably further in describing these qualitative responses, noting especially the formation of purple pigments in plants grown on sterilized soils as well as changes in the character of the root system.

With respect to seed germination, aside from rate of germination, it may first be noted that many seeds, especially seeds of leguminous plants, apparently become much more easily over-run with fungi when germinated on heated soils. This fact is probably due to the absorption of the favorable property for fungus growth from the heated soil solution. In soils heated to 250°C., especially in those which are most toxic upon heating, the tip of the radicle may often become browned or blackened from contact with the surface of the heated soil. Lettuce seeds on the most toxic heated soils frequently germinate in an abnormal manner, in that the cotyledons emerge from the seed coat before the radicle. In other cases the radicle may make a long spindly growth, seemingly in an attempt to grow away from the toxic medium. The lack of formation of root hairs as compared with the abundant formation on unheated soils is especially noticeable at some stages of germination and growth on heated soils. If the seeds are allowed to continue germination on the surface of heated soils, it may often be noted that the roots instead of penetrating into the soil, grow along the surface of the soil. When roots are, however, forced to grow into certain sterilized soils in order to maintain the life of the plant, the roots may be much retarded in growth and may become short and stubby, without root hairs, frequently discolored as a whole or in local areas, or deeply split radially, partly decayed and sometimes entirely killed. This is especially true in sterilized soils grown to plants under sterile

conditions. It seems reasonable to suppose, therefore, that the retarded early growth of plants on heated soils may in some instances be due in part to the failure of the root system to penetrate the soil with the consequent reduction of their functions, rather than to the absorption of the toxic principles of the plant. This point is illustrated in plate 5, figure 1 showing the retardation of the root system of tomatoes grown at different soil temperatures. That toxic principles are absorbed is shown by the cases of "chemical" injury obtained. That absorption of toxic properties occurs is also shown in seed germination. Certain seeds, of which lettuce may be cited as an example, behave very peculiarly on the most toxic of the heated soils, heated soil extracts, or products of dry distillation of soils as already referred to. This is shown especially by the swelling of the seed to two or three times its normal size, together with a decided change in color of the interior seed coat to a greenish black. The seed in such cases is practically always killed and sometimes bursts the outer seed coat due to internal pressure. The inner seed coat is very tense and hard, and upon pressure, bursts, exuding a droplet of clear liquid. This reaction with lettuce seed is sufficiently characteristic to make it valuable as a qualitative test of toxic agents and will be referred to as such in the consideration of the nature of toxic properties produced in heated soils.

The qualitative responses of growing plants to heated soils are numerous and varied in type. Russell and Petherbridge (64) have made some observations on this, especially with tomatoes. With regard to the formation of a purple pigment the impression is left that the pigmentation is characteristic on heated soils particularly. This does seem to be the case, however. Tomatoes grown on a wide variety of soils and under several different conditions may produce excessive purple pigmentation. Soils low in fertility, or cold soils especially, seemingly respond in a similar manner to heated soils in pigment production. Purple pigmentation on heated soils is not peculiar to tomatoes alone, but seems to be a response more or less common to other plants, being especially noticeable in cabbage and lettuce, as far as observed in these experiments. Although pigmentation sometimes occurs on heated soils in case of tomatoes, it is not necessarily associated with heating. It may occur in the early plant growth, and finally disappear in the later growth; its persistence when present, apparently is in proportion to the unfavorableness of the soil for plant growth.

Another type of color reaction which has been noted in the case of tomatoes, but which may be associated with stimulated as well as retarded growth, is a yellowish green mottling or mosaic appearance of the leaves resembling to some extent the Mosaic disease but usually without malformations (plate 6, fig. A). This abnormality also usually disappears in a short time and is apparently associated with diminished activity of the chlorophyll bodies due to the absorption of a toxic substance fron the soil. In other plants such as tobacco, mottling may not occur but rather a uniform yellowing, especially at the leaf tips or margins.

The most marked effect of heated soil on plant growth, however, is that which has been termed "chemical" injury. This is characterized by distinct spotting of the leaves or lesions on the stem, sometimes the latter occurring at the surface of the soil and resembling the damping-off disease. These spots or lesions often become confluent and the entire leaf or plant may wither and die. In some instances this action has been observed to become localized just below the terminal bud, resulting in the death of the latter and consequently the premature branching of the plant. In other instances "chemical" injury appears in the early stages of growth of the plant with the result that they may be killed outright or remain for weeks or months without making appreciable growth. Where the action has not been too severe, however, the plants may entirely recover and produce greater yield than in unheated soil. On the other hand this type of injury has been observed in plants which apparently made normal growth during the early stages of growth but finally showed symptoms of chemical injury in a late stage of growth (plate 5, fig. 2). The first signs in such cases are sometimes in the veins and midribs of the leaf, and are exhibited by the browning of the veins and curling of the leaves or at other times by spots which become confluent resulting in the entire leaf drying and dropping from the stalk. This condition has all the appearances of a disease caused by a parasitic organism. On account of the fact that it is not always associated with retardation, it appears that it is not necessarily the result of a product formed during heating of the soil but may be the result of a substance developed subsequent to heating. It is, however, also probable that the explanation of this response occurring several weeks after heating the soil may be due to the occurrence of certain environmental conditions favorable for its production or manifestation, as, for instance, modified soil temperature or moisture conditions. This conclusion is supported by the previously mentioned soil temperature experiments and by the observation that overwatering heated soils may increase this type of toxic action.

Similar "chemical" injury which may be mistaken for diseases of parasitic origin have been found to occur upon several other plants grown on heated soils, particularly upon soybeans, cow peas, and tobacco. In the case of soybeans, the browning of the veins of the leaf is especially striking. If this action affects the leaves when they are young and in a rapidly growing condition, it results in leaf curling (plate 6, fig. E). The condition is similar to that sometimes occurring on tomatoes. The spotting of the leaves of soybeans on heated soil usually occurs on the leaf margins and is seemingly associated with the rapid transpiration at these points (plate 6, fig. D). In the case of cow peas, the spotting is far less distinct on the leaves, the spots being much smaller, usually not larger than a pin head, and raised rather than sunken (plate 6, fig. F). In the case of tobacco, the spots resemble very much certain leaf-spots occurring in the field, some of which are of bacterial origin and others probably of a non-parasitic nature (plate 6, fig. C).

These qualitative responses are much more marked in some soils than in others. They may occur in a wide range of soil types and have seemingly no relation to the nitrogen content of the soil. They are influenced by environmental conditions of the soil especially, but they do not appear to be necessarily associated with degree of toxicity as represented by retardation of plant growth in heated soils, in view of the fact that they may appear on plants benefited in their growth by heated soils as well as on plants which are retarded. The relation of soil temperature to this toxic action has been especially demonstrated in the case of tomatoes and tobacco. It was found that "chemical" injury to tomatoes and tobacco on heated soils usually occurred at soil temperatures between 15° and 25°C. and not at higher or lower temperatures. In later experiments, however, it became evident that this injury might occur at lower or higher temperatures if other conditions, such as greatly increased moisture content of the soil, occurred. All observations point toward the fact that a fairly delicate relation exists between the extent of "chemical" injury and the balance between soil moisture and soil temperature. In the acid sand (lime requirement 9.38 tons per acre) the "chemical" injury was marked following sterilization. If neutralized with calcium carbonate before sterilization, this injurious action did not occur. All evidence has gone to show that this toxic action is most marked in acid soils following sterilization.

Other minor qualitative responses of plants to heated soils occur, such as increased tendency toward branching in the case of soybeans. Under greenhouse conditions at least, the soybeans normally drop most of the lateral buds as well as the lower leaves and the plants tend to grow tall and spindly rather than short and bushy. Although this condition may be especially associated with infertile soils, it is seemingly also associated with reduced light. In heated soils, however, these lateral buds do not fall off as readily in most instances but persist and produce short stout leafy branches deep green in color, even in cases of marked retardation due to toxic action.

Some mention is to be found in the literature of increased susceptibility of plants to disease as a result of being grown on heated soil. Wilson (78), for instance, reports wheat rust (*Puccinia graminis*) and wheat mildew (*Erysiphe graminis*) more serious on weakened plants grown on soils heated to high temperatures, than on more vigorous plants grown on soil heated to lower temperatures. The probable changes in susceptibility to disease is to be considered in connection with a separate paper on the use of heated soils in phytopathological research.

The development of fungi in heated soils

That certain fungi grow very well on burned-over areas has probably been noted, with only passing comment, for a very long time. Tacke (74), however, in studying the effect of the heating of soil on the solubility of nitrogen, noted

that fungi grew very well on the extracts of heated soils and believed that this was due to the excellent nitrogen supply present. Kurzwelly (39) reported that various species of fungi not living in soil before sterilization flourish vigorously afterwards. Kosaroff (35) noted, especially, the development of *Pyronema confluens* on heated soils where it did not occur on unheated soils. He concluded that the extraordinary development of this fungus on heated soil was not due to the resistance of the spores of the fungus to the sterilizing action but rather to the destruction of a substance toxic to the development of *Pyronema* in ordinary soils. Seaver and Clark (67, 68) began a series of investigations on the development of *Pyronema omphiloides* on heated soils, in an attempt to explain the reason for this behavior and inclined toward the belief that the beneficial action upon fungi was due to the increased concentration of the food supply.

In the open and especially in the greenhouse in the presence of air-borne spores, the heated soils rapidly became covered with a thick mat of *Pyronema confluens* (Tul.) or *Pyronema omphaloides* (Bull.), Fuckel. Under favorable environmental conditions the soil may on the other hand become overrun with various ordinary molds, especially those of *Penicillium* and *Mucor*. It appears, however, that heated soils are considerably more favorable for *Pyronema* than for *Penicillium* and other fungi, but no more favorable for the latter than for other forms. The common occurrence of their spores in the air, probably explains the presence of certain fungi on the heated soils in many instances. Where other fungi, such as *Rhizoctonia*, *Pythium*, *Thielavia*, *Fusarium*, and other organisms commonly inhabiting soils are reinoculated into heated soils, they apparently find it a very much more favorable medium for growth than unheated soils.

Although no detailed experimental work concerning the relation of fungi to heated soils was carried on in connection with the experiments of the writer, yet considerable observational data are at hand, which are seemingly in some instances contradictory to some of the conclusions of the above named authors. The relation of the following factors in regard to the growth of fungi upon heated soils has been especially noted: temperature to which soil is heated, influence of soil type, influence of soil reaction, length of time of heating, growth on soil extracts, and the effect of soil temperature and moisture on fungus growth in heated soils together with certain correlations between fungus growth and various other factors studied. The observations have shown that the growth of fungi in soils heated to different temperatures is best at about 250°C., the growth either occurring later or being less profuse or entirely absent in soils heated to higher or lower temperatures (plate 7, fig. 1). The range for the development of *Pyronema* appears to be from about 100°C. to 350°C., temperatures above or below this usually giving no growth whatever. This is seemingly true for practically all soils studied, but much more marked in some than in others. It should be noted at once in this connection that there is a close correlation between this growth and various other phe-

nomena studied in soils heated to different temperatures, i.e., toxicity to seed germination, early plant growth, and beneficial action to late plant growth. It may be, therefore, that the property which is favorable for the development of *Pyronema* is the same which is toxic to seed germination and early plant growth as believed by Seaver and Clark (68), but no further evidence of this theory could be found; while on the other hand some contradictory evidence has been secured.

The extent to which fungi, especially *Pyronema*, develops varies greatly for different soils when heated to 250°C., it being on the whole greater on those soils high in organic matter and less in those low in organic matter, although no definite proportion in this respect has been established. Comparing the development of *Pyronema* on the various soils used, it was usually found to be most profuse on the peat and least profuse on the Norfolk sand. It was interesting to note that *Pyronema* in one instance grew most profusely on peat soil heated to 250°C. which was so toxic to tobacco and other plants transplanted into it, that they were killed in a few days. It has been observed, however, that *Pyronema* has made an appreciable growth upon red clay and upon practically pure sand heated to 110°C. The relatively low concentration of the soil solution in these cases raised the question as to the importance of concentration of soil solutions in connection with fungus growth on these soils, and this point will be discussed briefly in a later paragraph. The reaction of the soil within wide ranges (lime requirement of 9.38 tons per acre to one with corresponding alkalinity) seemed to have no influence on the rate of growth of *Pyronema*. In regard to the influence of moisture supply on the development of *Pyronema* on heated soils, it was found that the best development was on soils kept at one-half saturation. Somewhat less growth occurred on soil kept at one-fourth saturation, followed by that at three-fourths saturation with relatively poor growth. No growth was obtained on soil kept at full saturation. No growth, of course, is obtained on soil which is air dry, but it should be especially noted that the property favorable to the development of *Pyronema* may be kept indefinitely in air dry soils, whereas it is relatively rapidly lost in moist or wet soils.

The time of heating the soil has some influence on the growth of *Pyronema*, it being found for instance to occur first on soil heated to 115°C. for 160 minutes, and then appearing gradually on soils heated for 80, 40, 20, and 10 minutes. In this case no correlation seemed to exist with the growth of tomatoes on this soil. It has generally been found that an appreciable increase in concentration of soil solution and ammonia occurs on continued heating of soil at 115°C. The development of *Pyronema* upon the surface of soils maintained at different constant temperatures offers some interesting suggestions. At temperatures above 28°C. no growth of *Pyronema* has been observed. At temperatures ranging between 20° and 28°C., *Pyronema* usually appeared soon after the heated soil had been moistened, fruited, and then disappeared in a relatively short period of time. Finally growth appeared gradually upon

the soil at lower temperatures, and at temperatures of 10° and 15°C., the fungus persisted and grew for several weeks, an occurrence which is relatively uncommon under ordinary conditions. The late appearance and slower growth at the lower temperatures is, no doubt, partly due to a temperature relation effecting the organism itself, but it is also reasonable to suppose that the persistence of *Pyronema* at these lower temperatures is due also to the maintenance of the favorable property in the heated soil, and its early disappearance at the higher temperatures is due to the rapid loss of this favorable property for its growth.

That heated soils are also favorable to the growth of bacterial organisms is evidenced by the fact that extracts of heated soils left exposed to the air or inoculated with bacteria soon become teeming with organisms. Lodge and Smith (41) in experiments with decoctions of sterilized and unsterilized soil found that bacteria may or may not be favored in growth by the sterilized soil decoction, depending upon the nature of the soil. The extract of soil heated to 250°C. has been repeatedly noted in my experiments to be more favorable to the growth of bacterial organisms than extracts of the same soil heated to higher or lower temperatures. This has also been shown by making up soil extracts with 2 per cent agar and inoculating tubed slants with pure cultures of *Bacillus subtilis*. The growth in heated soil extract agars was best in the 250°C. extract and poorer in the higher or lower temperature extracts. The growth of *Penicillium* in sterilized soil extracts from heated soils was approximately in the same proportion and in the following order: 250°C. extract best, 200°, 150°, 100°, 350°, 50°, and unheated poorest. As noted by Seaver and Clark, it is practically impossible to keep soil extracts from being very strongly invaded with lower microörganisms without keeping them under sterile conditions.

The nature of the toxic and beneficial substances produced on heating

The earliest theories in regard to the cause of the injurious action of heated soils were largely founded on the effect of heat on the physical properties of the soil, particularly on the destruction of vegetable matter in burning, although Davy (12) as early as 1819 did not agree with these theories. Arends (1) believed that bad results follow burning due to exhaustion of fertility. Struckmann (73) suggested that certain inorganic substances may be produced which are injurious. Dietrich (13) was first to carefully observe the injurious action of heated soils, and concluded that there was a poison formed from the alteration of the organic matter, and that according to the amount of this poison formed and the sensitiveness of the plant to it, either the injurious or the beneficial action predominated. Where the plants were less susceptible to the poison, there was observed an increased absorption of soluble nitrogen by the plants.

Schulze (66) found a harmful effect of steam sterilization of soil which he attributed to increased solubility of the humus. The effect was found to vary

with different soils and plants, and to be more marked in early stages of plant growth.

Pickering (50) in 1908 published the first of a series of four papers on the action of heated soil on seed germination and plant growth and the changes occurring in the heated soils. His work was especially concerned with the toxic action of the heated soil, although no special attempt was made to determine any definite injurious compound. Extensive studies were made particularly concerning the relation of the increased solubility of organic and inorganic material in the soil due to heating. These papers are of sufficient importance in connection with the investigations reported here to briefly review each separately. Pickering's conclusions were more or less modified in each succeeding paper, however, and it is somewhat difficult to determine to which points most significance should be attached in a brief review.

Pickering's first paper (50) brought out some very interesting conclusions which are further extended in the following papers. The unfavorable effect on seed germination was found to be proportional to the content of organic matter when different heated soils were compared. In general the "richer" and more favorable the soil for germination before heating, the more inhibitory it becomes after heating. He believed that the destruction of the inhibitory substance in heated soil, as a result of added extracts of unheated soil, is due to a process of oxidation. No appreciable destruction of the toxic property resulted when soil was kept for several months at a low temperature in a moderately dry condition. At higher temperatures, however, in presence of moisture, some of the inhibitory properties were lost, probably through oxidation. The inhibitory substance was found not to be of an acid reaction. The retarding effect could not be explained by alteration of bacterial flora in the soil as the alteration extends progressively beyond that necessary to destroy all bacteria. The maximum of toxic effects to seed germination was found to be produced on heating to 200°C., although in a second paper (51) 250°C. was said to be the optimum temperature for toxicity. In this paper Pickering also concludes, contrary to his first paper, that the "poverty" or "richness" of a soil under ordinary conditions bears no relation to its behavior when heated. There appeared to be no connection between the total nitrogen in the soil and the extent to which it was altered by heat, and the inhibitory substance produced was not apparently the same in all cases. Pickering's third paper (52) concludes, relative to the nature of the toxic agent, that the increase in soluble matter produced by heating a soil and the accompanying toxic properties detrimental to the germination of seeds are gradually reduced by exposing the soils in a moist condition to the air, even under aseptic conditions, but are not reduced when the soils are kept moist in the absence of air. The destruction of the toxic substance is therefore thought to be due to oxidation.

In a fourth paper (53) concerning plant growth in heated soils, Pickering concludes that the substances favorable for plant growth and the substances

unfavorable for plant growth in heated soils are of a different nature. The toxic action on plant growth is believed to disappear under the influence of oxidation as it does in the case of seed germination as noted above. Whether or not the toxic agent to seed germination and plant growth is identical cannot be settled but it is provisionally assumed that they are the same. As a result of growing different kinds of plants in heated soils, Pickering concludes grasses are more resistant to the toxic action than non-grasses.

Schreiner and Lathrop (65) heated soils to 135°C. under steam pressure and found an increase in various organic constituents not ordinarily considered in questions of soil fertility. Some of these were beneficial to plant growth and one in particular, dihydroxystearic acid, was found to be harmful to plant growth. These authors are inclined to explain the injurious action produced on sterilized or heated soils to the formation of this toxin and the beneficial action to the formation of other organic compounds. Although the writer attempted to isolate dihydroxystearic acid from the most toxic of the heated soils studied without success, the main objection to this theory in connection with explaining the response of plants to highly heated soils seems to be that it is highly probable that this compound having a low dissociation temperature, would be practically destroyed before a temperature of 250°C. is reached.

The objection to Fletcher's theory (18) that heated soils do not produce toxins, which delay the germination of seeds, but that this delay is caused by decreased imbibition of water from strong solutions of heated soils seems to be contradicted by the observations made in connection with the experiments described in this paper where it was found that certain seeds actually imbibed more water than in normal germination.

Russell and Petherbridge (64) present a considerable amount of data bearing upon the retardation and stimulation of seed germination in soils heated to low temperatures and treated with antiseptics. They made no study, however, of this problem in particular, but were concerned mainly with the beneficial action of sterilized soils upon plant growth. These authors disagree with Pickering as to the chemical nature of the toxic substance and state that they could not find proof that the harmful effect of heated soils on germinating seeds passed off after a time. That ammonia might be the toxic agent produced was suggested, but they could find no analytical data to show that any relation existed between it and the amount of retardation or acceleration to seed germination.

Lyon and Bizzell (42) have also made a careful study of the substances produced in steamed soils injurious to plant growth. They found that the time required for the various soils to recover from this injurious action was, with one exception, in the order of their relative productiveness. Steaming was found to increase the soluble nitrogenous compounds and also phosphoric acid. The nitrates were reduced to nitrites and ammonia, but most of the ammonia was formed from organic matter. They concluded that the toxic

substance produced is a controlling factor in productivity of steamed soils. Apparently, however, they do not suggest anything further as to the chemical nature of the injurious property.

Practically all the results of previous investigations on the nature of the toxic agent in heated soils have been based on results secured on heating below the "critical" temperature. It seemed to the writer, therefore, that a detailed study of soil heated to the optimum for the production of the injurious action might lead to conclusions more satisfactory than those secured from lower temperatures of heating. That the substance toxic to seed germination increases with the rise in temperature up to about 250°C., and that on further heating, the toxic property is gradually lessened until it has entirely disappeared before a temperature of 500°C. is reached, has repeatedly been found to be true. It is reasonable to assume, at least, that at about 250°C. a balance is reached between the maximum production and maximum retention of the toxic substance by the heated soil. Whether the substance is destroyed at temperatures above 250°C. or merely volatilized is not exactly clear. There is some reason to presume, however, that the toxic substance is gradually produced in increasing amounts up to 250°C. and beyond, but that it is rapidly volatilized above 250°C.

Water extracts of heated soils

Water extractions were made of soils heated to various temperatures and the comparative rate of seed germination obtained. The results for water extracts of Waukesha silt loam are shown in table 21, where it may be seen that the toxic property is at least partly soluble in water. Considerable variation is shown in the germination percentages after 24 and 42 hours. This may be due in part to experimental error, but is also seemingly a further indication of similar conditions repeatedly noted in which certain soil heated to 50°C. are more injurious to germination than unheated soil or soils heated to 100°C. This condition has been found to recur too often to be considered merely accidental and yet no explanation can be offered for it at this time as it appears to bear no consistent relation to any of the changes in the soil which have been studied. The germination after 66 hours, however, as shown in table 21 does suggest that the amount of toxin soluble in water is in proportion to that in the soil itself. In some cases the extraction of a soil heated from 200°C. to 300°C. with an equal weight of water yields an extract considerably more toxic than the soil itself. Such marked toxicity is not usually obtained, however, but it is not improbable that the increased injurious action of the extract, beyond that of the soil itself, is due to the separation of the toxin from the ameliorating effect of the absorptive power of the soil.

In order to arrive at some approximation of the proportion of the toxic matter extracted with water in repeated extractions of the same soil, the experi-

ment detailed in table 22 was performed, two different soils being used. The results show that a large proportion of the toxicity was removed at the first extraction and that the second and third extractions with water yielded considerably less of the toxic property. Since the property toxic to seed germi-

TABLE 21

Relative rate of germination of seed on water extracts of soil heated to different temperatures; Waukesha silt loam; lettuce seed

TREATMENT	GERMINATION AFTER					TOTAL
	24 hours	42 hours	66 hours	90 hours	114 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Not heated	30	93	99			99
Heated to 50°C.	3	68	98	99		99
Heated to 100°C.	24	90	99	100		100
Heated to 150°C.	1	7	74	98	99	99
Heated to 200°C.	0	13	50	79	92	92
Heated to 250°C.	0	1	17	76	97	97
Heated to 300°C.	34	93	99	100		100
Heated to 350°C.	2	41	100			100

TABLE 22

The toxicity of repeated water extractions of soil heated to 250°C. on seed germination; lettuce seed

SOIL	TREATMENT	EXTRACTION	GERMINATION AFTER					TOTAL
			42 hours	66 hours	90 hours	114 hours	138 hours	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam	Check	First	77	96	97	98		98
	Heated	First	1	2	17	76	78	79
		Second	11	40	59	74		75
		Third	57	80	85	92	93	94
Virgin sandy loam	Check	First	98	99				100
	Heated	First	2	7	26	69	76	77
		Second	9	50	65	87	89	89
		Third	45	82	86	93	94	94

nation is readily soluble in water, further study upon heated soil extracts was made.

That this toxic principle is extractable from all types of heated soils is shown in table 23, although it is not proportional to the toxicity exhibited by the soils themselves. Peat and muck and especially the fine sandy loam, for instance, ordinarily do not exhibit as great a toxicity to seed germination as Waukesha silt loam. The extracts of these first-named soils, after hav-

ing been heated, are, however, considerably more toxic than the heated soils themselves, and slightly more toxic than the extract of Waukesha silt loam. This is especially significant when it is remembered that the concentration of the soil solution used is far less than that actually existing in the soils themselves. The absorptive capacity of the soil, then, has seemingly a most profound influence not only upon the total toxicity exhibited by a soil but also upon its relative toxicity, and this is apparently roughly correlated with the total amount of organic matter in the soil.

TABLE 23

Relative rate of germination of seed in water extracts of different soils heated to 250°C.; lettuce seed

SOIL	GERMINATION AFTER							
	42 hours	66 hours	90 hours	114 hours	138 hours	152 hours	176 hours	200 hours
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Distilled water	60	78	82	87	90			90
Peat	0	0	0	0	0	2	6	9
Muck	0	0	2	5	20	27	35	39
Waukesha silt loam	0	4	20	44	65	72	76	81
Fine sandy loam	1	3	9	26	53	63	69	75
Virgin sandy loam	2	8	32	53	76	80	82	82
Clay	2	10	19	33	61	70	73	78
Norfolk sand	21	51	73	87	89	91	93	

TABLE 24

Seed germination in distillates from extract of heated soil. Waukesha silt loam heated to 250°C.; lettuce seed

FRACTION	GERMINATION AFTER						TOTAL
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Check (distilled water)	89	99					
First (gaseous)	2	4	14	36	44	45	45+
Second	1	3	13	22	48	63	66
Third	4	11	38	63	84	90	92
Non-volatilized	0	5	31	80	89	92	92

Further evidence upon the nature of the toxic property is obtained by distilling the soil extract. A rough fractionation was made of a water extract of Waukesha silt loam heated to 250°C. The fraction (table 24) which came over before the soil solution came to the boiling point showed almost as much toxicity to seed germination as the second portion and considerably more than the third, which came over after boiling. That all the toxic substance was not volatile is shown by an active toxic principle apparently still remaining in the distillation flask.

Further evidence of the volatility of the toxic principle in heated soil was procured in several different ways. Heating soil in closed vessels usually produced more properties toxic to seed germination than heating in open vessels. Drawing a current of air through the soil into water while heating yielded toxic properties in the water. Drawing air through the dry soil after heating also removed some of the toxic principle.

It has been hoped that the difference in reaction of seeds of different species of plants would give some clue to the nature of the injurious action. Several experiments were therefore planned for the purpose of comparing the response of different seeds to heated soils and to known chemical substances.

The difference in response of seeds of various kinds to the toxic action of heated soil has already been referred to. In an effort to determine if this reaction bore any relation to the genetical relationship of the various groups of plants, a germination test was performed using several different species of the same family in comparison with various species of other families (tables 25 and 26). The results indicate that a considerable degree of correlation exists in this regard. The *Gramineae* are characteristically "resistant" to the toxic action as are also the *Cucurbitaceae.* The *Solanaceae* and *Leguminoseae* are, however, characteristically quite "susceptible" to the injurious action, although not so much so as lettuce seed. The data at hand are not sufficient to satisfactorily establish this rule even for the families mentioned and cannot, of course, be said to apply to other families of plants although there is a suggestion and natural expectation that this may be true. It is interesting to note here that oats and rye showed some degree of stimulation on Waukesha silt loam heated to 250°C., an occurrence which illustrates the striking resistance of these species to the injurious action as compared with lettuce or clover. On the assumption that different seeds respond differently to different chemicals, it seemed reasonable, therefore, that if a considerable degree of correlation could be found between the germination of different seeds on heated soil and their germination in the presence of certain pure chemical substances of known strength, it would be possible to arrive at some conclusion regarding the composition of the injurious product produced on heated soil.

Production of ammonia in heated soils

Of the substances commonly present in soils, which are at the same time toxic in relatively very small amounts and which have further more been frequently found to be toxic to plants in soil (14), ammonia is probably the most common. Various investigators have shown a small initial production of ammonia in soil on sterilization. Hérbert (24) as early as 1889 noted ammonia production in soil on heating to 100°C., while Kelley and McGeorge (30) and the results reported in this paper as well as that of others have shown large increases on heating to higher temperatures. It was logical, therefore, to closely examine the relation of ammonia to the toxic action of the

TABLE 25

Relative rate of germination of seeds of different families of plants on heated soil; Waukesha silt loam soil heated to 250°C.

FAMILY AND SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		45 hours	79 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Gramineae								
Rye	None	76	85	87				87
	Heated	81	85	86				86
Wheat	None	57	96	98				98
	Heated	31	91	98				98
Barley	None	70	97	99				99
	Heated	51	95	98				98
Oats	None	8	75	97				98
	Heated	10	88	98				99
Corn	None	8	28	55	89	94	96	97
	Heated	3	15	46	74	89	94	98
Cucurbitaceae								
Cucumber	None	35	82	87	88			88
	Heated	21	74	85	88			89
Squash	None	36	81	91				91
	Heated	4	38	63	71	72	73	81
Pumpkin	None	66	87	96	98			98
	Heated	11	51	82	93	94	95	95
Muskmelon	None	25	51	72	73	76	77	77
	Heated	4	13	34	40	50	66	69
Watermelon	None			7	32	49	69	82
	Heated			2	17	31	56	85
Solanaceae								
Nicotiana rustica	None		81	85	86			86
	Heated		17	60	72	77	81	84
Egg plant	None		16	38	59	66	69	71
	Heated		0	0	1	8	36	72
Pepper	None		1	3	6	12	34	51
	Heated		0	0	0	3	16	55
Tomato	None		3	32	50	57	63	69
	Heated		0	0	7	34	49	64
Datura	None			21	56	69	75	76
	Heated			0	0	14	42	

TABLE 26

Relative rate of germination of seeds of the legume family on heated soil; Waukesha silt loam heated to 250°C.

SEED	SOIL TREATMENT	GERMINATION AFTER							TOTAL
		45 hours	66 hours	90 hours	114 hours	138 hours	162 hours	210 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Red clover	None	41	80	86					86
	Heated	0	2	4	7	11	14	21	53
Alfalfa	None	22	43	51	52				52
	Heated	1	6	11	15	20	25	34	41
Bur clover	None	21	27	31	32				32
	Heated	8	12	13	15	15	16		18
Tapary bean	None	13	77	84	89	91			91
	Heated	0	52	80	89	91			91
Crimson clover	None		10	14	15				15*
	Heated		0	0	1				1*
Goats rue	None		6	10	12	13			13*
	Heated		0	3	6	10	11	12	12*
Robinia	None		3	6	9	17	20	25	25
	Heated		0	2	5	14	19	23	25
Hairy vetch	None		1	9	13	15	16	17	17*
	Heated		1	3	12	16	17		18*
Geuge clover	None		2	11	21	27	29	31	31*
	Heated		0	0	0	1	2	3	3*
Birdsfoot clover	None			8	10	11	12		12*
	Heated			0	2				2*
Astragalus	None			8	19	30	34	40	46*
	Heated			0	0	1	1	2	5*
Peas	None				20	38	77	82	82
	Heated				6	21	58	74	74

* Seeds molded and failure to germinate may be partly due to this, but the seeds were also low in germinating capacity.

heated soil. That this increase is appreciable on heating to 115°C. and very striking on heating to 250°C. is shown by four separate determinations for two different soils in table 27.

Two soils were now taken and heated to temperatures ranging from 50° to 350°C. and the amount of ammonia produced determined. Here it was found

that on the whole there was a gradual increase in ammonia on heating to temperatures up to 250°C. On heating to higher temperatures, the ammonia content was reduced (table 28). The close correlation between the ammonia content on heating to different temperatures, and the toxicity to seed germination is evident from the data presented. The reduced ammonia content on heating to 60°C. has some semblance of an error in determinations, but on the other hand its frequent occurrence in the experiments together with certain

TABLE 27

Increase of ammonia in soils heated to 115°C. and 250°C.

SOIL	TREATMENT	NITROGEN AS AMMONIA IN 100 GM. SOIL					AVERAGE INCREASE
		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Average	
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Muck	Not heated	4.20	6.93	3.36	6.58	5.28	
	Heated 115°C.	6.72	8.32	8.26		7.77	47
	Heated 250°C.	31.15	31.01	25.06	30.10	29.33	455
Waukesha silt loam	Not heated	1.96	1.68	1.82	2.9	2.09	
	Heated 115°C.	3.50	3.78	4.34		3.87	85
	Heated 250°C.	18.48	13.72	15.40	17.0	16.15	672

TABLE 28

Increase of ammonia in soils heated to different temperatures

SOIL TREATMENT	NITROGEN AS AMMONIA IN 100 GM. SOIL			
	Muck		Waukesha silt loam	
	Single determination	Increase	Average of duplicates	Increase
	mgm.	*per cent*	*mgm.*	*per cent*
Not heated	3.36		1.75	
Heated to 60°C.	1.54	−118	1.40	−25
Heated to 115°C.	8.26	149	4.06	132
Heated to 200°C.	14.42	329	9.52	444
Heated to 250°C.	25.06	646	14.56	732
Heated to 300°C.	5.88	66	7.32	318
Heated to 350°C.	3.92	16	3.36	92

irregularities of seed germination and plant growth on some soils, as already referred to, leads to the conclusion that some correlation may exist even here. This may be illustrated further by results secured on heating soil for various lengths of time (table 29) where not only the ammonia content was lowered on heating 10 and 20 minutes, but also the concentration of the soil solution compared with longer periods of heating, and these changes were accompanied by reduced germination on the soil but increased germination in the soil extracts.

On comparing the amount of ammonia produced in muck and Waukesha silt loam soil on heating them to different temperatures (table 28), it is found that the ammonia content is uniformly higher in the muck soil than in the Waukesha silt loam. Muck soil is, however, never as toxic to seed germination as Waukesha silt loam when heated to the same temperature. That this increase in ammonia occurs in all soils used and is at its maximum at about 250°C. has been frequently shown in these experiments. The increase on heating to 250°C. has usually ranged from 100 to 1,000 per cent for the different soils. No results such as reported by Potter and Snyder (55) i.e., reduced ammonia content on heating peat to 200°C., have been obtained. No great uniformity in results has been secured in toxicity produced on heating the same soil to a certain temperature at different times owing to the variation in the actual amount of heat applied which variation seems unavoidable where automatically regulated ovens can not be used in all cases. The relative results where different soils were heated at the same time in the autoclaves or ovens, have, on the other hand, been remarkably uniform. It should now be stated that the ammonia content of different soils heated to the same temperature seemingly bears no relation to their toxicity to seed germination. This conclusion is illustrated in table 30 showing the results secured on heating different soils to 115°C., although it might be shown better by soils heated to 250°C. The results are somewhat confusing because of the fact that different soils behave differently towards seed germination in their natural or unheated condition. This is especially marked in the case of Sparta sand, and may be due to a toxic agent which is normally present in this soil, and which is more or less common in all soils, since seed germination is apparently always somewhat retarded on unheated soils or their extracts as compared to germination in pure water on filter paper. In any case, heating of the soil to certain temperatures appears usually to reduce the natural retarding influence to seed germination.

TABLE 29

Effect of heating soil at 115°C. for different lengths of time; Waukesha silt loam; lettuce seed

TIME OF HEATING	NITROGEN AS NH_3 IN 100 GM. SOIL	LOWERING OF FREEZING POINT	GERMINATION					
			On soil after			On soil extract after		
			42 hours	66 hours	90 hours	24 hours	48 hours	72 hours
minutes	*mgm.*	*°C.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
None	3.0	0.007	97	99	100	0	83	99
10	2.1	0.004	54	98	100	4	84	100
20	2.8	0.001	49	93	100	11	83	97
40	3.5	0.015	67	98	99	2	59	100
80	4.1	0.018	69	97	98	3	71	95
160	4.6	0.026	62	93	97	12	78	94
Distilled water						48	96	98

If heated and unheated soils are extracted with water and the ammonia determined in the extracts, it will be found that a relatively high amount of ammonia exists in the heated soil extract as compared with the unheated soil extracts (table 31). This suggests that the absorptive capacity of the soil for ammonia has been reduced by the heating. With weak hydrochloric acid more ammonia can be extracted, but this extract containing more ammonia is less toxic than the water extract. The toxic substance is then apparently

TABLE 30

Effect of heating different soils to 115°C. in autoclave on ammonia content and on rate of seed germination; lettuce seed

SOIL	TREATMENT	NITROGEN AS NH_3 IN 100 GM. SOIL	INCREASE IN AMMONIA	GERMINATION AFTER			TOTAL
				24 hours	42 hours	66 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Peat	Check	11.7		43	99		99
	Heated	15.0	28.2	0	48	70	75
Muck	Check	4.2		68	99		99
	Heated	7.4	76.2	0	88	98	98
Waukesha silt loam	Check	3.8		3	87	95	96
	Heated	4.8	26.3	0	80	96	98
Virgin sandy loam	Check	3.0		45	100		100
	Heated	4.1	36.6	5	98	99	99
Fine sandy loam	Check	2.4		56	98		98
	Heated	3.4	41.6	2	93	98	98
Sparta sand	Check	1.9		0	54	80	91
	Heated	3.2	68.4	2	72	94	96
Norfolk sand	Check	1.6		16	88	91	92
	Heated	2.2	37.5	0	49	83	95
Red clay	Check	0.5		7	89	97	97
	Heated	0.5	0.0	1	94	100	100

neutralized by the acid, or in some manner rendered less injurious. This experiment suggests at once both the possible relation of free ammonia to the toxicity of heated soils and the relation of the absorptive capacity of the soil for ammonia to the injurious action.

It is fairly clear, nevertheless, from the data presented thus far that ammonia determinations of the soil as such cannot lead to any clear conception of the relation of ammonia to seed germination. This is largely because of the fact that the toxicity of a chemical substance in a soil is in proportion

to the absorptive capacity of that soil for the substance in question. The great absorptive power of soil for ammonia is well known. It is, in fact, recognized in farm practice that the use of ammoniacal fertilizers on soils low in absorptive capacity may be productive of toxic action on plant growth (14). Due consideration must be given this matter, therefore, in relation to heated soils. Several angles of the problem present themselves for consideration. Soils of widely differing absorptive capacities have been used in the experiments. The absorptive capacity of each soil is influenced to a considerable extent by the degree of heat applied. That this is not a simple relation is indicated by the marked alteration of most soils as regards absorptive capacity for water when heated (by moist heat especially) to temperatures of about

TABLE 31

Ammonia in extracts of heated soil and seed germination in distillates from extracts with magnesium oxide; heating to 250°C. and extracted with distilled water and 2 per cent HCl; lettuce seed*

SOIL	TREATMENT	NITROGEN AS NH_3 IN EXTRACT OF 100 GM. SOIL	GERMINATION AFTER					TOTAL
			24 hours	42 hours	66 hours	90 hours	114 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Waukesha silt loam.....	Check, water extract	0.2	11	88	97	98		98
	Heated, water extract	3.2	0	17	56	60	62	66
	Heated, acid extract	5.1	0	38	74	77	81	87
Virgin sandy loam.....	Check, water extract	0.35	39	97	98	99	100	100
	Heated, water extract	3.7	0	7	16	21	28	38
	Heated, acid extract	5.5	0	16	25	27	41	50
Distilled water....			62	99				100

* The ammonia was determined from a composite of three washings of the soil in equal weights of solvent after standing 24 hours with frequent shaking in each case. The lettuce seed was germinated in a part of about 25 cc. of distillate collected in 5 cc. of water.

80 to 115°C., compared with the very rapid absorption of water in soils heated to high temperatures. This may not be a problem of simple absorption, however, but rather one of capillarity. In general the heating of soil reduces its capacity of absorption, although considerable disagreement may be found in literature in regard to this, due most likely to differences noted in capillarity. If decreased absorption is the rule then in addition to the variation in different soils, it is reasonable to expect that the toxic property produced in a heated soil will vary in one direction in proportion to its natural absorptive capacity, and in the other, in proportion to the degree of heating. The method of solving this problem, however, does not seemingly lie in attempting to obtain correlation between the absorptive capacities of the various soils under different conditions, but in studying the correlation in respect to seed germina-

tion where definite and varying amounts of a toxic agent are added to the soil itself; the main difficulty encountered in this procedure being, however, that it offers no satisfactory direct solution of the problem as between heated and unheated soils.

Two preliminary experiments were made to arrive at a rough approximation of the absorptive capacity of different soils for ammonia, as measured by its toxicity to seed germination, and the response of different seeds to different amounts of ammonia in soils. It will be seen from table 32 that the smallest amounts of ammonia added had no appreciable effect on either the muck or silt loam soil, but that higher amounts stimulated germination and that the highest application retarded germination considerably in Waukesha silt loam. Quartz sand with very low absorptive capacity showed retardation at the lowest percentage of ammonia used. In the same way, where different seeds were used on one soil (table 33), certain seeds showed slight stimulation where the smallest amount of ammonia used was present, whereas others showed retardation. Rye and buckwheat were more resistant to the injurious action of ammonia than clover or lettuce seed. Table 34 illustrates the same facts with a greater variety of seeds. Rye, seemingly the most resistant, was followed in order by wheat, buckwheat, and flax. Garden-cress was the most susceptible (lettuce was not used in this experiment), followed by clover, cabbage, and tomatoes. Beans, cucumbers, tomatoes, and Datura showed an intermediate degree of resistance. If now, the germination of these seeds on Waukesha silt loam heated to 250°C. is compared with the germination on soil to which ammonium hydroxide has been added, considerable similarity will be noted (table 35). With the strength of ammonia used in this experiment, we find that in the case of all seeds, the soils treated with ammonia were more toxic than the heated soils. However, in the case of flax which is very resistant to the toxic action of heated soil, the seeds were practically all killed by the soil to which ammonia had been added. Wheat, buckwheat, and cucumber, which are quite resistant to the toxicity of heated soil, respond proportionally in the same manner to ammonia, and lettuce, garden-cress, clover, and cabbage, which are quite susceptible, respond in a similar way to ammonia. The difference may be noted that in the case of those seeds susceptible to ammonia, practically all the seeds were killed, whereas in the heated soil, the large majority gradually germinated.

The seeming failure of flax seed in this experiment to fall in line with expectations led to a more detailed experiment to determine if this seed would correlate itself in some measure with the other resistant seeds with respect to germination on soil to which ammonia had been added. Garden-cress was chosen as a check seed, as it had shown itself susceptible to ammonia in the previous experiment, and was at the same time quite susceptible to the toxicity of heated soil. Different amounts of ammonia were added to soil both as ammonium hydroxide and as ammonium carbonate. The amount of ammonia in these and in the heated and unheated samples was determined

TABLE 32

Action of different amounts of ammonia added to different soils on seed germination; cabbage seed

SOIL	NH_3 ADDED	GERMINATION AFTER						TOTAL
		24 hours	32 hours	48 hours	60 hours	78 hours	102 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Muck	None	5	38	69	70	73	73	73
	0.02	6	30	64	70	71	73	73
	0.025	14	37	65	70	71	71	71
	0.05	8	32	61	65	68	70	71
	0.1	7	29	65	67	72	72	72
Waukesha silt loam	None	12	28	55	63	64	65	71
	0.02	8	23	68	70	72	74	74
	0.025	6	37	66	71	74	75	75
	0.05	14	40	68	73	76	78	79
	0.1	1	15	47	63	67	71	73
Quartz sand	None	13	32	60	70	73	80	82
	0.02	0	0	19	33	47	62	69
	0.025	0	0	0	0	0	.0	0
	0.05	0	0	0	0	0	0	0
	0.1	0	0	0	0	0	0	0

TABLE 33

Action of various amounts of ammonia added to Waukesha silt loam soil on germination of different seeds

SEED	NH_3 TO DRY SOIL	GERMINATION AFTER								TOTAL
		24 hours	32 hours	46 hours	70 hours	94 hours	118 hours	142 hours	166 hours	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	74	85	90						90
	0.04	78	89	96						96
	0.1	3	44	79	82	86				86
	0.2	0	0	0	0	0	0	0	0	0
Clover	None	15	69	88	89					89
	0.04	13	56	83	89	90				90
	0.1	0	0	0	0	3				
	0.2	0	0	0	0	0	0	0	0	
Rye	None	57	86	96	97					97
	0.04	58	90	95						95
	0.1	16	70	91	92					92
	0.2	0	0	0	0	0	0	0	0	
Buckwheat	None	0	0	12	35	56	63	67	68	
	0.04	0	0	9	38	50	61	64	64	
	0.1	0	0	13	43	52	63	65	67	
	0.2	0	0	0	0	0	0	0	0	

TABLE 34

Relative rate of germination of different seeds on soil treated with ammonia; Waukesha silt loam treated with 0.05 gm. NH_3 (25 cc. of 0.2 per cent NH_3 solution) to 50 gm. of soil

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wheat	None	55	97	98				98
	NH_3	51	97	98				98
Rye	None	66	85	88				88
	NH_3	70	81	86				87
Garden cress	None	21	38	53	62	63	65	65
	NH_3	0	3	9	13	17	27	50
Bean	None	43	88	91				92
	NH_3	23	94	97				97
Clover	None	63	82	86				
	NH_3	5	35	45	55	58		60
Flax	None	98	99					99
	NH_3	74	85	88				89
Cabbage	None	47	62	66	67	68		69
	NH_3	12	38	49	52	58		66
Cucumber	None	41	84	85				85
	NH_3	8	76	82	84			85
Buckwheat	None		8	25	36	54	60	63
	NH_3		4	21	28	40	50	61
Tomato	None		18	44	55	58	61	66
	NH_3		2	28	53	58	61	69
Datura	None			43	65	68	69	83
	NH_3			34	55	57	60	97

by the ordinary method in order to aid further in the correlation of facts (table 36). From the data it may be seen that at the end of 54 hours and thereafter, garden-cress is considerably more susceptible to heated soil than flax seed. Where 62 mgm. of ammonia were present, the cress seed was very strikingly retarded and flax seed only slightly. A similar proportion apparently exists where other strengths of ammonia were used. It is believed, therefore, that the results obtained warrant the tentative conclusion that as far as the response of different seeds to the toxic action of ammonia is concerned, a high degree of correlation exists between it and the toxic action on these seeds produced by heating the soil.

TABLE 35

Relative rate of germination of different seeds on soil treated with ammonia and heated to 250°C. Waukesha silt loam 0.075 gm. NH_3 (25 cc. of 0.3 per cent NH_3 solution) to 50 gm. soil*

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	53	76	80	81			81
	Heated	0	1	10	18	23	39	58
	Ammonia	1	2	4	4	4	5	7
Flax	None	95						95
	Heated	91	96	97				97
	Ammonia	0	1					1
Wheat	None	53	93	96				96
	Heated	49	91	96				96
	Ammonia	25	73	87	88	90		90
Cabbage	None	4	28	36	42	45	46	46
	Heated	6	3	6	8	18	27	44
	Ammonia	0	0	0	1	1	1	1
Buckwheat	None	53	93	94	95			95
	Heated	50	90	93	94			94
	Ammonia	40	88	90	91			92
Garden cress	None	27	65	71	75	76		76
	Heated	12	26	29	30	38	53	65
	Ammonia	0	0	0	0	0	0	0
Clover	None	47	78	85				85
	Heated	3	21	42	50	61	71	80
	Ammonia	0	3	5				5
Tomato	None	0	22	86	91	92	93	93
	Heated	0	0	21	71	86	88	92
	Ammonia	0	0	38	64	71	73	81
Cucumber	None	0	61	84	86	87		87
	Heated	0	55	82	87	87	89	89
	Ammonia	0	31	62	70	72	76	79

* Contained 14.7 mgm. NH_3 per 100 gm. soil.

Referring further to table 36, however, it may be seen that in the case of both cress and flax greater toxicity to seed germination existed on heated soil with only 20 mgm. of ammonia present than was true when 32 mgm. of ammonia which had been added to the soil directly were present. The supposition that the difference here is due to the reduced absorptive capacity

of the heated soil for ammonia seems reasonable. An attempt was made to test this theory by adding ammonia to soil heated to 250°C. and 350°C. as compared with equal amounts added to unheated soil. For some reason the addition of ammonia to soil heated to 250°C. did not increase the toxicity up to the expectations; the absorbing power for ammonia was apparently still large. On the soil heated to 350°C. however, the absorbing power was greatly reduced as indicated by increased toxicity. Unfortunately no further attempt was made to check up this apparent discrepancy and it must be assumed that if ammonia is responsible for toxicity in the heated soil, that various chemical (61) as well as physical properties of the soil influence its manifestation.

TABLE 36

Germination of seed on Waukesha silt loam soil treated with ammonia as ammonium hydroxide and ammonium carbonate as compared with heated soil

SEED	SOIL TREATMENT	AMMONIA AS NITROGEN IN 100 GM. SOIL*	GERMINATION AFTER				TOTAL
			30 hours	54 hours	78 hours	102 hours	
		mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Garden cress....	None	3.8	13	55	85	86	88
	Heated to 115°C.	3.8	11	46	67	72	84
	Heated to 250°C.	20.4	0	8	38	41	79
	NH_4OH 0.1 per cent†	32.0	2	16	58	70	71
	NH_4OH 0.2 per cent	62.2	0	1	6	9	53
	NH_4OH 0.3 per cent	91.0	0	0	0	0	0
	NH_4HCO_3 0.1 per cent	44.0	1	10	46	69	88
	NH_4HCO_3 0.2 per cent	87.8	0	0	0	0	0
	NH_4HCO_3 0.3 per cent	122.4	0	0	0	0	0
Flax.....	None	3.8	77	96			97
	Heated to 115°C.	3.8	61	94	97	98	98
	Heated to 250°C.	20.4	6	90	96		97
	NH_4OH 0.1 per cent†	32.0	72	94	95		96
	NH_4OH 0.2 per cent	62.0	42	79	88	91	92
	NH_4OH 0.3 per cent	91.0	0	4	7	8	8
	NH_4HCO_3 0.1 per cent	44.0	35	93	96		96
	NH_4HCO_3 0.2 per cent	87.8	0	7	12	13	13
	NH_4HCO_3 0.3 per cent	122.4	0	0	0	0	0

* Represents amount recoverable by magnesium oxide method.

† Calculated per cent solutions applied.

That ammonia exists in the heated soil as an absorbed gas seems improbable. Under such conditions it would be gradually lost through areation even in dry soil. The toxic property is exceedingly stable, however, in perfectly dry soil. The readiness with which it becomes active and is lost in moist soils indicates a very unstable condition in the presence of moisture and

suggests the similarity of the product to such an unstable salt as ammonium carbonate as shown in table 36. For this reason ammonia was added to the soil in proportionate amounts both as carbonate and hydroxide. The result was practically the same as shown by retarded seed germination. It may be assumed that the loss of toxicity in heated soils is purely a chemical question and is due to the breaking down of ammonium salts and the loss of the toxic radical as in the case of ammonium carbonate. But assuming further that ammonia is actually the toxic agent, a difficulty is at once encountered. If ordinary sterilized soils are either naturally or artificially inoculated with normal soil flora, the ammonia content as determined by the ordinary methods shows a gradual increase, together with a reduced toxicity to seed germination. The increased ammonification in sterilized soils has been repeatedly shown by various investigators and has been previously referred to here. The results presented in table 37 show that a reduced toxicity to seed germination has occurred in the face of increased ammonia content during a period

TABLE 37

Germination of lettuce seed on virgin sandy loam after increase in ammonia due to sterilization and re-inoculation

TREATMENT	NITROGEN AS NH_3 IN 100 GM. SOIL	GERMINATION AFTER		TOTAL
		26 hours	42 hours	
	mgm.	*per cent*	*per cent*	*per cent*
Not heated; moist 14 days	4.7	92	99	99
Heated to 115°C.; moist 14 days	11.9	75	97	99
Not heated (kept dry)	2.8	88	99	99
Heated to 115°C.* (just before test)	3.7	11	94	97

* Dry.

of 14 days of exposure to the air. The results indicate a reduced toxicity due to, or in close conjunction with bacterial activity favoring ammonification, and experiments were therefore later undertaken to determine in how far ammonification, or bacterial activity as such, reduced the toxic action of heated soils.

A theory assuming ammonia to be the toxic agent produced in heated soils would appear hopeless here were it not for other evidence. In order to maintain the theory at this point, various phenomena occurring in the fixation of ammonia in soil may be resorted to, not only to explain reduced toxicity due to changed form, but also to show that these forms of ammonia are easily reversible. Assuming that ammonia is the toxic agent, it is possible that it may be fixed or changed, chemically, biologically, or physically (43) into less toxic but unstable compounds which are again readily broken down to ammonia by the relatively violent method of determination of ammonia by distillation with magnesium oxide as used in these experiments. On the other hand,

this method may be quite satisfactory for actual ammonia determinations in heated soils where such decomposition has already occurred, and is perhaps not advanced much further by the distillation process.

Even though a very satisfactory correlation could be obtained between seed germination on soil to which ammonia is added and on heated soil, this would not be sufficient ground to prove the relation of ammonia to the toxic action of heated soils. The objection can be made that many seeds may react in a similar manner to two or more toxic chemical agents. This subject has been considered only in a preliminary way and as far as could be found, the literature upon this point is neither extensive nor very helpful. In a preliminary experiment testing various radically different chemicals such as acids, the strong alkalies, various organic bases, and formaldehyde on seed germination, it was found that the qualitative differences in reaction were sufficiently great in most instances to warrant the conclusion that certain chemicals gave fairly distinctive reactions with certain seeds. The qualitative response of seeds to ammonia has been previously mentioned. The swollen, blackened condition of certain seeds, especially lettuce, exposed to weak strengths of ammonia was not in any case approached by exposure to other chemicals used. That ammonia causes increased imbibition of water by seeds due probably to its action upon the permeability of the seed coat, has been described especially by Brown (7) and it is believed that the swelling of seeds treated with ammonia in these experiments is due to the same cause.

In order to get more evidence upon the similarity of the action of heated soil, its extracts or distillates, and of ammonia upon seeds, histological studies were undertaken. Lettuce seeds which were swollen and killed by heated soil extracts and ammonia were fixed with mercuric chloride, imbedded in paraffin, sectioned and stained, and compared with sections of normal seeds. In both instances the inner seed coat was found to be greatly distended from the cotyledons, which were apparently not greatly affected in size. The color change in the seed was seemingly due to the deposition of a new substance between the cotyledons and the inner seed coat. This substance in sections was of a greenish purple color and seemed to be made up of bodies of a fairly definite structure, and usually more or less globular in form. Although the study of these sections was not carried out in any great detail, the similarity of these bodies to those described by Darwin (11) in roots injured by ammonia was especially noticeable and interesting.

That other substances besides ammonia may react in a similar manner with lettuce seed is not questioned but as yet no chemical substance which is likely to be present in heated soil, seems to produce this reaction. The results on rate of seed germination with formaldehyde as compared with ammonia are given in table 38. This may serve to illustrate the point that different chemical substances may be expected to behave differently in their effect upon the rate of seed germination also. The data indicate that ammonia at the strength used was relatively more toxic than the heated soil in the

case of the four seeds treated. On the other hand, with three seeds formaldehyde was more toxic than ammonia, while in the other case, i.e., the cucumber seed, it was relatively less toxic than ammonia. This evidence seems to illustrate the point that seeds do not on the whole, react in a similar manner to toxic chemical agents.

The distinctive odor of heated soils, especially of the products of dry distillation, point strongly towards certain organic bases such as pyridine, piperedine, or quinoline as being active agents in toxicity to seed

TABLE 38

Comparison of the relative rate of germination of different seeds on sterilized soil and soil treated with ammonia and formaldehyde; Waukesha silt loam heated to 115°C.; treated with 0.3 per cent NH_3 and 1–200 formalin

SEED	SOIL TREATMENT	GERMINATION AFTER						TOTAL
		42 hours	66 hours	90 hours	114 hours	138 hours	162 hours	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lettuce	None	83	92	94				95
	Heated	8	40	67	83			84
	Ammonia	10	19	22	23	30		30
	Formaldehyde	0	1	11	33	45	47	47
Flax	None	97	98					98
	Heated	92	94	95				95
	Ammonia	2	6	9	11	12	17	17
	Formaldehyde	0	0	0	0	0		0
Wheat	None	30	93	97				97
	Heated	47	96	97				97
	Ammonia	18	93	98	99			99
	Formaldehyde	8	69	95	98			98
Cucumber	None	5	65	82	84	85	86	86
	Heated	10	71	86	87	88		88
	Ammonia	0	31	76	83	84	86	86
	Formaldehyde	0	53	81	86	87	91	91

germination or plant growth. In the absence of any satisfactory qualitative or quantitative method of determination of these bases in the presence of ammonia, considerable significance can be attached to the qualitative effect of ammonia upon lettuce seed as a means of determination. Pyridine, piperidine, or quinoline, and other related compounds do not cause the increase in pigmentation of lettuce seed characteristic of ammonia, although some may produce swelling of the seed. The response of lettuce seed to ammonia and to highly toxic products of heated soil are, however, apparently identical.

Relation of soil flora to reduced toxicity

The most extensive work upon the nature and loss of toxicity of heated soils has been that of Pickering (50—53). His conception of the toxic property produced in heated soil as concluded in practically all of his papers, is that it is organic and nitrogenous in nature and is destroyed by oxidation. Some reasons for believing that the disappearance of the toxic principle is not due to a simple chemical oxidation process, but that it is due to the activity of soil microörganisms have already been mentioned. In order to obtain further data upon this subject an experiment was performed in which soil was stored for a period of about two months under various conditions as far as aeration, moisture, and activity of microörganisms were concerned. The soil was stored in wide-mouthed bottles of 3 liters capacity and placed in a horizontal position in order to expose as much of the soil surface as possible to the air. These bottles were carefully plugged with cotton. Where it was desired to exclude air, bottles of only sufficient size to hold the soil were used and these were tightly corked and sealed with paraffin. Virgin sandy loam soil was heated to 200°C., thoroughly mixed, and the equivalent of 600 gm. of air-dried soil added to each of 13 containers. In the unheated checks 600 gm. of air-dried soil was used. The soils to be moistened were then watered with an equal quanity of water, plugged as desired and the heated series sterilized in the autoclave for about 1½ hours at 15 pounds pressure. Certain of the soils were then inoculated with various microörganisms while others were kept sterile. The treatment of each soil, together with the results, are shown in table 39. Although the tests were run in triplicates in some instances they are not included in the table. The data on the amount of ammonia present and the toxicity to lettuce seed were taken after 55 days of storage. Transfers of soil were made to determine the nature of the flora present or of possible contamination. The results are believed to be very significant in indicating the nature of the loss of the toxicity in particular. It will be noted that the initial production of ammonia on heating to 200°C., as shown in the unaltered soils, ranges around 13 mgm., three to four times the amount in the normal unheated soil. In the presence of microörganisms and moisture, the ammonia content has appreciably increased, due to the activity of either bacteria or fungi, either in the presence or absence of good aeration. The importance of various fungi as ammonifiers in the soil has been well reviewed and described by McLean and Wilson (44). As was to be expected, the microörganisms had no influence on the dry soil. In the sterile moist soil whether in the presence or absence of good aeration, no appreciable change in ammonia occurred. Comparing the toxicity to seed germination with the ammonia determinations it may be seen that the reduced toxicity is approximately directly proportional to the increased content of ammonia. In other words, the toxicity has been reduced by the microörganisms where present and active as measured by increased ammonification, but has not been reduced appre-

ciably in the absence of these organisms, and in the presence of otherwise identical conditions. Such a biological explanation of the disappearance of toxic compounds from the soil has been recently suggested by Robbins (59). In no case, during the time of storage given and at the temperature and other environmental conditions afforded, was the toxicity completely reduced. It should be remembered, however, that this heated soil represents a much higher degree of toxicity than is ordinarily secured in soil sterilization. The conditions for free access of air were of course not the best even though a cotton-plugged wide-mouthed bottle was used with the soil layer hardly

TABLE 39

Influence of various manners of storage of heated soil on ammonia content and rate of seed germination; virgin sandy loam heated to 200°C.; stored 55 days; lettuce seed

CULTURE NUMBER	TREATMENT						NITROGEN AS AMMONIA IN 100 GM. SOIL	GERMINATION AFTER							TOTAL
	Heated	Sterilized	Inoculated with	Aeration	Moisture	Sterile cultures		27 hours	39 hours	55 hours	76 hours	118 hours	160 hours	232 hours	
							mm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	+	+		+	+	+	13.9	0	0	2	26	52	80	82	
3	+	+	5 cc. soil extract	+	+		20.9	13	68	94	97	97	99	99	99
5	+	+	Aspergillus	+	+		15.8	0	2	10	45	62	88	96	96
6	+	+	Pyronema	+	+		19.5	0	5	40	80	88	94	94	94
9	+	+		−	+	+	13.3	0	0	1	13	26	57	80	96
11	+	+	5 cc. soil extract	−	+		19.8	0	5	11	25	39	79	81	95
7	+	+		+	−	+	13.2	0	0	3	12	23	48	88	
8	+	+	1 gm. dry soil	+	−		13.2	0	0	1	12	41	78	85	94
12	+	+	1 gm. dry soil	−	−		12.6	0	0	1	6	31	65	78	88
13	+	+		−	−	+	13.6	0	0	2	7	35	54	79	92
14	−	−		+	−		3.5	89	98	99	100				100
17	−	−		+	+		4.3	95	97	99	99				99
15	−	−		−	+		7.6	90	97	98	98				98
16	−	−		−	−		3.6	84	93	95	97				97

more than an inch in thickness at its maximum depth. This does not influence the result, however, in showing the comparative importance of bacterial and "mold" activity and aeration alone in reducing toxicity. The least loss of toxicity was obtained in the soil kept sterile, aerated, and dry and the greatest loss in the soil inoculated with normal soil flora in the presence of aeration and moisture. The efficiency of *Pyronema* in reducing the toxicity is especially interesting, and indicates that this fungus so commonly occurring on heated soils may serve a very useful purpose in reducing their toxicity to higher plants.

It may be argued again from this experiment that the increase in ammonia content together with decreased toxicity is evidence against ammonia having

anything to do with toxicity to seed germination. We may assume, however, that ammonia in the presence of microörganisms is fixed or exists in various unstable stages of fixation which are no longer toxic but which may again be reduced to ammonia on boiling with magnesium oxide, and is hence recorded as ammonia present in the soil, though it no longer plays a part in influencing seed germination. That various transition products of ammonia are changed to ammonia by sterilization has been found by Nikitinsky (45) and others.

It is evident that the toxic substance produced in heated soils is soluble in water. Therefore assuming that ammonia is the toxic agent, more ammonia should be found to be soluble in water where its soil content is highest. This was found not to be the case, only about 3.3 mgm. being extractable in the inoculated aerated moist soil, whereas 4.35 mgm. were found in the sterile aerated wet soil. In the inoculated not-aerated moist soil 5.35 mgm. were found to be present, this higher amount being due apparently either to reduced loss of ammonia from lack of aeration or to increased denitrification.

The differences in odor of the soils kept under these various conditions was very marked. Where no activity of microörganisms occurred in the heated soils the odor was the same as that of the heated soils immediately after heating, whether kept dry or moist, aerated or not-aerated. Where microörganisms had been active in the aerated cultures all the odor of heated soils had apparently disappeared, the only detectable odor being similar to that of ordinary soil, except in the case of the Pyronema and Aspergillus cultures where a "moldy" odor was very evident. In the case of the not-aerated moist inoculated cultures, however, a very marked disagreeable odor, similar to that of fermenting manure was present, considerable hydrogen sulfide probably being present. The action of denitrifiers in the absence of aeration no doubt account for this condition. The results in table 39 together with other evidences obtained are believed sufficient to justify the conclusion that the loss of the toxic substance from heated soils is not a simple matter of oxidation proceeding even under aseptic conditions as concluded by Pickering, but is due rather to the activity of certain bacteria and fungi which become reintroduced into sterilized soils. That *Pyronema*, which is commonly found on sterilized soils is very efficient in this respect has been shown and indicates that its preference for heated soils over the unheated soils may be one of relation to ammoniacal nitrogen, just as other fungi, bacteria or green plants differ in forms of nitrogen preferred.

In studying the properties of the toxic or beneficial agent in the heated soils, it is evident that many advantages can be secured by separating the toxic property as much as possible from the body of the soil itself. This may be accomplished by extractions either with water or other solvents, or by means of collecting products driven off from the soil upon heating to different temperatures. This latter method is essentially dry distillation. It is believed that much important evidence secured in the future upon the subject of sterilized soils will be obtained through a more careful study of the

extracts or dry distillation products of heated soils. Aside from the work of Seaver and Clark (68), very little work has been done upon this phase of the problem.

When soil is heated to various temperatures and extracted with water, it becomes evident at once that the color of the soil extract increases gradually with the temperature of heating up to 250°C., while further heating decreases color until practically a colorless extract is obtained from soils heated to 350°C. This has been found to be true for all the soils used, the color varying from a very light tinge of straw color up to a very dark brown wine colored liquid at 250°C. The depth of color varies very much for the different soils, and is roughly in proportion to their content of organic matter. Peat soil extract usually gave the deepest color, whereas extracts of red clay or Norfolk sand showed very little color. The color is a fairly good indication of the toxicity of the extract to seed germination, though germination does not necessarily always decrease with increase in color, i.e., a slightly colored solution may favor seed germination, as for instance, that from certain soils heated to 100°C.

As has already been stated, the fungus development in extracts of heated soil usually increases with the temperature up to 250°C., falling off again at higher temperatures, and the extent of fungus growth can to some extent be correlated with the color of the soil extract as can toxicity to the growth of green plants. These colored solutions are readily decolorized by filtering through boneblack or by other decolorizing agents without apparently affecting their chemical composition. The color is also rapidly lost in the soil, due either to reabsorption or destruction, but may be kept quite indefinitely as bottled extracts, although some changes appear to occur.

Closely associated with color in soil is the odor which is generally quite closely correlated with the color. The odor varies considerably from different soils but is usually quite characteristic, ordinarily being rather pleasant but difficult at times to compare with known odors. Seaver and Clark (68) likened this odor to that of caramel and again to pyridine. The odor of pyridine has also been very characteristic in my extracts together with those of picoline and quinoline. These odors are, however, far less marked in the ordinary extracts than in the products of dry distillation.

Soil extracts toxic to seed germination, if stored in stoppered bottles, can be apparently indefinitely kept without loss of the toxic quality, although some change occurs, especially where no attempt is made to keep the extracts sterile. Even in sterile extracts, however, some precipitation of material seems to occur from the filtered solutions on standing, it being considerably greater with some soils than with others.

Dry distillation of soil

To get a more concentrated form of the toxic property than that which could be obtained by extraction with water, dry distillation was resorted to.

A large bomb holding about 16 pounds of Waukesha silt loam soil was made in such a way that it could be placed in a gas oven to be heated and at the same time permitting air to be drawn through the soil and passed through bottles containing various solutions. The air was drawn through the soil and solutions by means of a filter pump. When drawn into water, the products were found to be toxic to seed germination and slightly acid in character. When evaporated to dryness, however, it became evident that the toxic property as well as the acidity was lost, indicating their volatile nature. Drawing the distillate from heating soil through barium hydroxide or calcium hydroxide showed that enormous quantities of carbon dioxide were driven off. This suggested that the increased acidity in heated soils might be due to the carbonic acid formed, which is partly corroborated by the loss of the acidity of the distillate on boiling. On longer heating and at higher temperatures, however, the distillate in water becomes colored and strongly alkaline, and also takes on the strong pungent odors of picoline, pyridine, or piperidine, masking seemingly some traces of odor of ammonia. Qualitative tests for ammonia with Nessler's showed strong reaction. Two dry distillations were now run with Waukesha silt loam by heating it between 250° and 300°C., and drawing the distillate through a weak solution of hydrochloric acid for several hours. This distillate was then evaporated to dryness and a dark colored salt was secured. It was again taken up with water, decolorized with animal charcoal and finally clean white salt was secured, which in one case amounted to 3.15 gm. and in the other 3.92 gm. from 16 pounds of soil. This was shown to be ammonium chloride by quantitative determination in comparison with chemically pure ammonium chloride. The yield of ammonia in the extracted salt was very close to that of the known salt. Tests of seed germination in the soil after distillation showed it extremely toxic, indicating that only a portion of the toxic agent had been withdrawn. The distillate in water from long dry distillation at 200° to 300°C. is darker in color than that obtained from water extract of heated soils, and has some oily properties which, however, apparently disappear on standing. Its toxicity to seed germination is very great; most seeds are killed when sown on filter paper moistened with the extract. The distillate has been kept in bottles for two years without apparently losing much of its toxicity. Fungi do not grow in the most toxic of these.

If this distillate is redistilled and roughly fractionated, we find that a gaseous product is at first rapidily given off at a rather low temperature. This gaseous substance is very alkaline and very toxic, and qualitative tests show strong reactions for ammonia. The later fractions show less alkalinity, less ammonia, and less toxicity. Not all the alkalinity ammonia, or toxicity, however, can be removed by boiling, indicating that some stable compounds of ammonia or other toxic agents are present. It is also interesting to note that, although the first distillates are colorless, they finally become colored, as a probable result of other substances going over at low temperatures in

addition to ammonia. The characteristic odors also distill over, and this fact, of course, argues against drawing any satisfactory conclusion regarding the relation of the ammonia present to seed germination. In relation to the toxicity of these distillates to seed germination, it should be noted here again that the same distinct qualitative reaction occurs as previously described, i.e., the seeds placed in contact with it are first discolored, in the case of lettuce becoming greenish black, and secondly that the seeds, especially lettuce and clover, rapidly swell to often two or three times their original size, frequently bursting the seed coat. Similar but much less marked results were secured by drawing air through soil heated in the autoclave at 115°C.

With reference to the effect of ammonia upon seeds as observed in this connection, it may be well to mention briefly the observations of others of a similar nature. Pickering (51) also noted that on one heated soil (Takoma loam) seeds were turned black when placed upon it. Bokorny (4) especially has noted the marked toxicity of very small amounts of ammonia upon seeds, he having found usually that 0.01 per cent was very harmful. In a later paper this author makes a special point of the fact that the injurious nature of ammonia is due largely to the ease with which it is able to combine with the cell protoplasm. Brown (7) in studying the selective permeability of the covers of the seeds of *Hordeum vulgare* found that the velocity with which water is absorbed from solutions of ammonia is remarkable. Similar osmotic relations on the part of ammonia have been previously noted by others (49). It is believed that the reaction observed by Brown is similar to the one with which we have been dealing and explains the swelling of seeds on distillates from heated soils. It should be said in passing that the views presented in this respect have been partially suggested by Armstrong (2) in some work upon stimulation of plant growth. In working with ammonia, he concluded that it seems to be the most important active "natural" stimulant, and that carbon dioxide is also effective as a hormone. He finds ammonia stimulates plant growth in small amounts and that it is toxic in larger amounts. Referring to Russell's work (64) and that of his collaborators, Armstrong adds that two powerful hormones are present in sterilized soils, i.e., ammonia and carbon dioxide, the latter being produced by increased oxidation. He believes that increased growth can be ascribed in large part to these, and that retarded growth may be explained by the assumption that they are in excess and that the balance is probably a delicate one.

The Concentration of the soil solution

The importance of the concentration of the soil solution in heated soils as subscribed to especially by Seaver and Clark (68) and Fletcher (18) is such that it seemed advisable to obtain further data upon this subject in relation to the observed conditions in the experiments carried on by the writer. The works of Pickering (51), Seaver and Clark (68), Wilson (79), and Boyoucos

(6) and Koch (33) have all established the fact that increase in concentration of the soil solution occurs on heating soils. Their determinations, however, were not for the most part carried on with soils heated to sufficiently high temperatures to arrive at a maximum concentration, as found on heating to 250°C. The method used in the determinations reported in this paper have been simply that of determining the lowering of the freezing point in soil extracts by means of a Beckmann thermometer. Boyoucos's paper (6) describing a method of immersing the thermometer bulb directly into the soil came after my determinations on soil extracts had been made. The results with soil extracts, however, seem to be equally satisfactory where only comparative results are desired. The method of using soil extracts seemed to

TABLE 40

Lowering of the freezing point of extracts of different soils heated to different temperatures

SOIL	TREATMENT	DEPRESSION OF FREEZING POINT AVERAGE OF DUPLICATE DETERMINATIONS				AVERAGE
		1	2	3	4	
		°C.	°C.	°C.	°C.	°C.
Muck	Not heated	0.019	0.012	0.023	0.026	0.020
	Heated to 115°C.	0.031	0.035	0.033		0.033
	Heated to 250°C.	0.145	0.189	0.086	0.199	0.155
Waukesha silt loam	Not heated	0.009	0.013	0.020	0.021	0.016
	Heated to 115°C.	0.016	0.024	0.040	0.026	0.026
	Heated to 250°C.	0.032	0.056	0.073	0.079	0.060
Fine sandy loam	Not heated	0.027	0.013	0.011	0.012	0.016
	Heated to 115°C.	0.034	0.009	0.014	0.030	0.022
	Heated to 250°C.	0.074	0.044	0.047	0.052	0.054

possess one difficulty, however, namely that although the readings for any one series of solutions gave uniform and constant results, they usually did not compare well with readings made of other extracts from the same soil. Since this variation occurred in the unheated as well as in the heated soil, it is apparently not only due to differences in temperature of heating or time of heating but also to variations in degree of solution. Since certain soils filter with much more difficulty than others, especially those unheated or heated to a low temperature, force filtering was used in such cases, and it was thought that this or a small difference in time of extraction might explain the variation in results. This was shown by preliminary experiments, however, apparently not to be the case. The averaged results are, nevertheless, satisfactory for showing the increase in concentration of the soil solutions in different soils when heated to different temperatures. That such increased concentration is quite marked is shown by the results summarized in table 40. From this table as well as from table 41, it may be seen that the concentration of the

soil extract from muck soil heated to 250°C. is two or three times as great as that of Waukesha silt loam heated to the same temperature. This is not in proportion to the relative toxicity to seed germination or to plant growth and indicates that concentration apparently has little to do with the retarded germination as contended by Fletcher (18) or to toxic action on green plants as proposed by Seaver and Clark (68). Reference to table 41 will show a gradual increase in lowering of the freezing point in the two soils when heated

TABLE 41

Lowering of the freezing point of extracts of soils heated to various temperatures

TREATMENT	DEPRESSION OF FREEZING POINT		
	Muck soil	Waukesha silt loam I	Waukesha silt loam II
	°C.	°C.	°C.
Not heated	0.012	0.021	0.009
Heated to 50°C.	0.011	0.020	0.011
Heated to 100°C.	0.035	0.026	0.016
Heated to 150°C.			0.037
Heated to 200°C.	0.124	0.064	0.060
Heated to 250°C.	0.189	0.079	0.064
Heated to 300°C.	0.059	0.053	0.036
Heated to 350°C.	0.052	0.055	0.048

TABLE 42

Lowering of the freezing point of different soils on heating to approximately 250°C.

SOIL	DEPRESSION OF FREEZING POINT; AVERAGE OF DUPLICATE DETERMINATIONS		NITROGEN AS NH_3 IN 100 GM. HEATED SOIL
	Not heated	Heated	
	°C.	°C.	*mgm.*
Peat	0.212	0.636	66.1
Muck	0.020	0.181	25.9
Waukesha silt loam	0.017	0.082	12.8
Virgin sandy loam	0.016	0.071	9.0
Fine sandy loam	0.018	0.053	10.2
Red clay	0.018	0.031	2.3
Norfolk sand	0.004	0.021	4.4

to various temperatures up to 250°C. followed by a decrease when heated to 300°C. A slight increase again on heating to 350°C. seems to indicate that at this high temperature marked decomposition of inorganic material has begun, following the destruction of the soluble organic matter. From the data presented in the last tables, there is shown to be a considerable degree of correlation between concentration of soil solution and the temperature of heating as far as any one soil is concerned, which condition is in turn correlated with such other factors as degree of toxicity, favorableness to fungus growth,

amount of ammonia produced, and color and odor of soil extracts. There seems to be no correlation, however, between concentration of soil extracts and these factors when different soils are compared. Determinations of the

TABLE 43

Temperatures developed in Dewar flasks with germinating wheat in extracts of heated soil

EXPERIMENT NUMBER	SOLUTIONS	TEMPERATURE REACHED						AVERAGE TEMPERATURE
		At start	After 1 day	After 2 days	After 3 days	After 4 days	After 5 days	
	Fine sandy loam extract							
		°C.	°C.	°C.	°C.	°C.	°C.	°C.
1	Redistilled H_2O	19.1	29.5	44.3	44.8	41.6	39.3	36.4
	Not heated	20.4	28.1	40.4	42.0	40.8	37.7	34.9
	Heated to 115°C.	21.4	31.3	42.5	42.6	41.3	38.3	36.2
	Heated to 250°C.	21.2	26.6	37.1	37.8	35.4	32.6	31.8
2	Redistilled H_2O	18.2	35.0	41.0	41.8	39.8	36.0	35.3
	Not heated	20.0	31.0	37.0	36.5	33.0	29.0	31.1
	Heated to 115°C.	20.7	33.7	39.2	41.7	39.2	38.0	35.6
	Heated to 250°C.	19.3	26.8	32.3	29.6	25.8	23.3	26.2
3	Redistilled H_2O	18.5	25.8	36.0	32.0	29.0		28.3
	Not heated	19.2	23.5	25.0	22.0	20.6		22.1
	Heated to 115°C.	19.2	25.0	28.1	24.0	22.0		23.7
	Heated to 250°C.	19.2	23.8	26.0	22.8	21.0		22.6
	Waukesha silt loam extract							
1	Redistilled H_2O	18.2	31.0	40.2	42.0	42.0	40.0	35.6
	Not heated	20.0	21.3	31.0	27.5	24.0	22.5	24.4
	Heated to 115°C.	20.1	29.0	34.0	31.0	27.0	24.5	27.6
	Heated to 250°C.	20.0	27.0	33.1	30.0	26.0	23.2	26.5
2	Redistilled H_2O	18.8	28.7	42.2	44.2	47.2	38.8	36.6
	Not heated	20.0	25.0	35.0	37.0	34.0	31.0	30.3
	Heated to 115°C.	20.2	26.5	38.5	40.0	40.5	36.5	33.7
	Heated to 250°C.	20.2	27.1	38.0	39.6	38.8	36.2	33.3
3	Redistilled H_2O	18.1	27.0	35.2	32.0	28.5		28.1
	Not heated	20.1	27.4	29.5	25.5	22.5		25.0
	Heated to 115°C.	19.0	26.8	33.5	30.1	26.7		27.3
	Heated to 250°C.	19.3	24.8	32.3	28.1	24.5		25.8

concentration of soil extracts were made from seven widely different soils heated to 250°C. (table 42). A wide variation in concentration will be noted, and although this appears to bear some relation to the amount of ammonia produced on heating, it is considerably less correlated with the toxicity of these extracts to seed germination.

In this connection it may be worth while mentioning briefly another method used in studying the behavior of soil extracts, with the hope of arriving at some conclusion as to the nature of their action upon seeds. This method was essentially that followed by Darsie, Elliott, and Peirce (10) in their study of the germination power of seeds in Dewar flasks by means of recording the temperature of respiration. A weighed quantity of wheat was placed in thermos bottles and sterilized with formaldehyde solution, after which equal amounts of extracts of unheated soil, soil heated to 115°C. and 250°C. was poured in the flasks. Redistilled water was used as checks. The thermometers were inserted through cotton plugs and into the wheat. The relatively small temperature differences secured are due largely to the fact that wheat, although cheap and convenient for this purpose, is relatively resistant to the action of heated soil extracts. If more susceptible seed had been used more marked results would no doubt have been secured. Some of the results are, however, presented in table 43 and serve to show the regularity of the results secured, in addition to corroborating results secured in actual seed germination tests. Comparing the average temperatures, it will be at once noted that the extract of an unheated soil is considerably less favorable to germination than pure water. Heating to 115°C., however, either destroys this toxicity in part or stimulates germination. Extracts of soil heated to 250°C. cause retardation of germination in wheat as measured by rise of temperature in thermos bottles and compared with the action of extracts of unheated soil.

Relation of toxic and beneficial properties.

It has been shown in this paper that a fairly marked correlation exists between seed germination and the toxic or beneficial action to plant growth when the same soil is heated to different temperatures, and that these are in turn correlated with the amount of ammonia present, as well as with the concentration of the soil solution. There is a strong presumption, therefore, that the property which causes simple retardation to plant growth is the same as the one which causes retarded seed germination. The property which causes "chemical" injury to plant growth, however, appears to be different from that causing retarded seed germination. Virgin sandy loam soil heated to 115°C. for 160 minutes, though extremely toxic to the growth of tomatoes reacted much the same as unheated soil in so far as lettuce or tomato seed germination is concerned. It is a difficult matter to prove definitely that the toxic agent causing retarded plant growth, and that retarding seed germination are the same, although the most reasonable presumption is that they are identical. There is, furthermore, seemingly no relation between the response of seeds to the toxic action and that of the early plant growth of any one plant. Lettuce which is for instance very susceptible to the toxicity of heated soils as far as seed germination is concerned, is relatively resistant

to the toxic agent during growth. Tomato plants, on the other hand, which are relatively susceptible during growth to the toxic properties, are more resistant in germination than lettuce seed. In general it may be said that no apparent striking correlation has been found to exist between seed germination and plant growth on soil heated to the same temperature. Traube (75) in working with acids and narcotics was also unable to find any constant relation between injury to germination and growth. In spite of this conclusion, however, one cannot fail to note exceptions. The seeds of the cereals are notably resistant to the agents toxic to germination, as are the cereal plants in their growth. Certain of the heated soils used, especially Waukesha silt loam, which is highly toxic to seed germination, is also relatively toxic to early plant growth, while others, especially fine sandy loam, not highly toxic to seed germination, are not very toxic to early plant growth.

TABLE 44

Production of ammonia in Waukesha silt loam soil heated to different temperatures on standing moist in greenhouse

SOIL TREATMENT	NITROGEN AS NH_3 IN 100 GM. DRY SOIL			TEST FOR NITRATES AFTER 18 DAYS
	Initial production	After 18 days "naturally" reinoculated	After 18 days artificially reinoculated	
	mgm.	*mgm.*	*mgm.*	
None		2.5	2.1	+
Heated to 50°C.		3.2	2.5	+ Strong
Heated to 100°C.	2.6	4.1	3.9	+ Strong
Heated to 150°C.	6.0	10.3	9.0	+
Heated to 200°C.	9.1	12.1	13.9	+
Heated to 250°C.	14.0	13.9	16.7	+ Weak
Heated to 300°C.	11.0	10.7	13.6	+ Weak
Heated to 350°C.	5.5	7.3	7.4	+ Weak

Regardless of the relation between the agents toxic to seed germination and plant growth, however, it may be well to consider the relation of the ammonia produced in the soil upon heating and subsequent to heating upon plant growth. All soils upon heating to different temperatures show usually an increasing toxicity to growth up to 250°C., which then falls rapidly and is practically nil at 350°C. While this is true for early growth, the final yields for most plants at least are usually greatest at 250°C., and fall off gradually at higher or lower temperatures of heating. With these facts only in mind, let us consider the ammonia relation in the soil under such conditions. Reference to table 44 shows a gradual increase in ammonia due to heating up to 250°C. but a falling off at higher temperatures. The period between the time of heating and the eighteenth day following may be taken as the average period during which toxicity to plant growth ordinarily occurs, since with many plants recovery from the toxic action may be well on the way after

3 weeks, especially at the lower temperatures of heating. Ammonia determinations were made after 18 days in both soils, in which inoculation with normal soil flora was made soon after cooling and where the soil was left to become "naturally" inoculated. These results show that after a period of 18 days, at least, soil heated to different temperatures and standing moist in the greenhouse increases rather than decreases in content of ammonia. These results were secured with an uncropped soil, and the question may arise as to what might be expected to happen on heated soils where crops are grown during this period of recovery. In an experiment Waukesha silt loam was heated to different temperatures ranging from 50°C. to 350°C. and planted to soybeans. It was found that the ammonia content was reduced to the greatest extent in the soils heated to the lowest temperatures (50°–150°C.) after 3 months. The soil heated to 200°C. and 250°C. retained the highest proportion of ammonia in relation to the amount produced by heating and these soils were still toxic to soybeans after 3 months and gave the smallest relative increase in yield. With radish, however, a more rapid reduction of ammonia content was produced, and the final yield was greatest on the soil heated to 250°C. The kind of crop grown apparently influences to a considerable extent the rate with which ammonia is removed from the soil, as well as the yield obtained from these soils. It is well known that heating the soil, at least to the higher temperatures, tends to reduce the nitrates present to very small amounts. Determinations made on Waukesha silt loam and muck by the colorimetric method showed that on heating to 250°C. the former was reduced from 2.75 mgm. nitrogen per 100 gm. soil to traces only, and in the latter cases from 2.28 mgm. to 1.19 mgm. Heating to 350°C. completely destroyed all nitrates. Qualitative determinations have shown that in highly heated soils increase in nitrates following heating is very slow, although it finally may become established. At any rate it is evident that the increased plant growth where it occurs relatively soon after heating soils to 250°–350°C., takes place in the presence of a greatly reduced supply of nitrate nitrogen but in an increased amount of ammoniacal nitrogen. Assuming ammonia to be present in sufficient amounts to be toxic to the early growth of plants, it is not difficult to conceive of the rapid recovery of plants resistant to its action owing to a decreasing amount (resulting from its use by the plant as well as to other changes) which finally reaches an optimum for the soil and for the plant, thus resulting in a very rapid increased growth followed by reduced rate of growth in the presence of a nitrogen supply below the optimum. For any one soil, the degree of toxicity, the time required for recovery, and the final increased yield are, then, largely dependent upon the temperature of heating and the susceptibility of the plants to the toxic (or beneficial) action.

That these soils respond to ammonia fertilization has been repeatedly noted and is illustrated in table 45 for tomatoes, where retardation was followed by marked increase in growth. The addition of ammonia as hydroxide to soils in different amounts, may, then, be regulated to such a point as to produce

an action quite similar to that resulting from heating the soils to various temperatures up to 250°C. Small amounts of ammonia show no retardation or stimulation, larger amounts very decided retardation. But the soils may finally recover completely from the injurious action and in the end show a markedly increased yield. Ammonium carbonate will produce similar results which in turn compare very well with the growth of tomatoes on soil heated to different temperatures (plate 7, fig. 2). Similar action may of course be obtained by other chemicals capable of being toxic, and later being volatilized or changed to a point where they may become a stimulant or a plant food. No clear case, however, of a response similar to the "chemical injury" of plants on heated soils has been obtained with pure chemicals.

That green plants are able to substitute ammoniacal nitrogen for nitrate nitrogen has been well established (26). It has also been shown that different plants vary in their ability to use ammoniacal nitrogen. Although the matter

TABLE 45

Yield of tomato vines on muck soil treated with different amounts of ammonia as ammonium carbonate

APPROXIMATE STRENGTH OF AMMONIA SOLUTION ADDED TO 2 KILOS SOIL	DRY WEIGHT	INCREASE	EARLY GROWTH
	gm.	*per cent*	
None	3.00		
0.1 per cent	8.75	191	Best; no retardation noted
0.2 per cent	13.30	243	Slight retardation
0.3 per cent	12.10	303	Marked retardation
0.4 per cent	11.25	275	First plants killed; reset; marked retardation

has not been investigated from this point of view, it seems reasonable to suppose that the differences in plants in their response to soil sterilization may be due in part to their capability of using ammoniacal nitrogen in the absence of nitrate nitrogen.

In this connection a study of the growth of plants on heated soils under aseptic conditions was made in order to determine the relation of the soil flora to the supply of plant food. Experiments in growing plants under sterile conditions in heated soil were started. Various forms of apparatus have been devised by investigators for growing plants under aseptic conditions, but these are ordinarily too complicated to permit the use of any considerable number of such structures. "Kellerman culture chambers" were finally selected for use in the experiments recorded here. These proved very satisfactory excepting for the fact that aeration is very poor under the conditions there afforded. It was found, therefore, that when a soil relatively toxic was heated or when plants relatively susceptible to toxic action were used, the time required for the plants to recover from the

injurious action was too long to permit a study of the beneficial action of the heated sterile soil on plant growth. Finally by selecting the least toxic soil (fine sandy loam), heating to 115°C. and planting to wheat, which is relatively resistant to the toxic action, it was found that fairly reliable results could be secured. That sterile conditions were obtained was demonstrated by transferring small bits of this soil with a sterile needle into bouillon tubes just before harvesting the crop. In the last experiment, the results of which are presented in table 46, one of the three flasks supposedly sterile had become infested with Penicillium but was apparently sterile insofar as bacteria were concerned. The increased growth on the reinoculated soil above that of the checks and sterile cultures is sufficient to be of considerable significance as indicating the relation of bacterial activity to the bene-

TABLE 46

Growth of wheat under sterile conditions as compared with unsterilized soil and sterilized and reinoculated soil

TREATMENT	NUMBER	DRY WEIGHT	AVERAGE	INCREASE
		gm.	*gm.*	*per cent*
No treatment	1	0.160		
	2	0.179		
	3	0.160	0.166	
Sterile soil	4	0.226		
	5	0.216	0.221	32.2
	6	*		
Sterilized and reinoculated	7	0.515		
	8	0.335		
	9	0.467	0.439	164.4

* This jar became accidentally infested with a fungus (Penicillium) and the yield was 0.482 gm.

ficial action of heated soils. The explanation of the results secured is not as simple as it may appear at first sight. Ordinarily it would be concluded that the increased growth on the reinoculated sterile soils is due almost entirely to increased bacterial activity in the sterilized soil as a result of new conditions which make this increased activity possible. The object of this experiment was to determine the relative importance of bacterial activity as compared with the direct chemical action of heat on soil in rendering food available for plants. It seems reasonable to assume that much of the increased plant growth on heated soils (especially of those heated to high temperatures) is due to direct chemical action in rendering plant food available. The results secured in growing plants under sterile conditions point quite strongly in this direction, since it was found that the plants grown in the sterile soil made an appreciable increase above the unheated checks in spite

of a toxic action sufficient to retard greatly the proper functioning of the root system. This condition was shown by the root systems in the sterile cultures which were very short, thick, small in total area, lacking in root hairs, penetrating only to shallow depths in the soil and generally unhealthy in appearance.

In the sterilized soils where reinoculation was made, the toxicity of the soil was apparently rapidly destroyed. The root system benefited thereby in quite a striking manner, being considerably better than that in the sterile checks or in the unheated soil (plate 8, fig. 2). Since all other factors were the same in the flasks except for the presence of soil organisms, it is evident that the destruction of the toxic substance was due in a large measure if not entirely, to their action. It seems extremely probable, therefore, that the increased growth in the reinoculated sterilized soil as compared with the sterile soil, was due in some considerable measure to the destruction of the toxic substance, which retarded the growth in sterile cultures but which was relatively rapidly destroyed or lost, or perhaps, even converted to plant food in the reinoculated soil cultures, and that the smaller growth in the sterile cultures was due as much to the toxic agent as to lack of increased food supply due to bacterial activity. This experiment illustrates further the important part played by soil organisms in benefiting sterilized soils due to the destruction of the toxic agent, making it possible for the plants to benefit sooner from the plant food rendered available as a result of the direct and indirect action of the heat upon the soil.

Conditions favoring development of microörganisms in heated soil

Turning to a consideration of the growth of fungi on heated soil, two possible hypotheses suggest themselves in the broadest sense. These are that a condition or substance unfavorable to fungus growth exists naturally in soil and is destroyed upon heating, or that new conditions or substances favorable to fungus growth are produced in heated soils. The former theory is essentially that of Kosaroff (35) who supposed that the soil contained certain toxins for fungi which are destroyed upon heating. Seaver and Clark (67, 68) have done a considerable amount of work in the support of the latter theory and conclude that the increased concentration of the soil solution favors fungus growth, which may at the same time be retarding in its influence upon the growth of green plants. Kosaroff's theory rests primarily upon the fact that the addition of the extract of a heated soil to an unheated soil will not permit of the growth of *Pyronema* upon the unheated soil. Seaver and Clark also obtained the same result. The writer was able, however, to obtain a good growth of *Pyronema* upon unheated soil by the addition of a heated soil extract following sterilization of the unheated soil by means of formaldehyde. It is highly improbable that a formalin drench (1–50) destroyed the soil toxin (and if it did it alone should permit of the growth of *Pyronema*),

but it did quite satisfactorily sterilize the soil for a time. It seems evident in view of the results previously described on the relation of the activity of soil microörganisms to the reduction of the toxic products produced in heated soil, that extracts of heated soils added to unheated soils are changed in character before the growth of *Pyronema* can establish itself. Although the formalin may have slightly increased the concentration of the soil solution (33) it is believed that its sterilizing properties were responsible for the changes produced which permitted the growth of *Pyronema* on heated soil extract added to unheated soil. This observation together with the findings of Seaver and Clark are believed sufficient to overthrow Kosaroff's arguments on the relation of soil toxins to the development of *Pyronema*.

That the concentration of food material as such plays an important part in the growth of fungi on heated soil as suggested by Seaver and Clark (67) seems doubtful in view of the following facts: the concentration of certain heated soils on which *Pyronema* grows readily may be considerably less than that of certain unheated soils or soils heated to 350°C. on which *Pyronema* will not establish itself. This is illustrated by the fact that *Pyronema* will make a good growth upon heated red clay or Norfolk sand or even relatively pure sand in which the concentration of the soil solution is relatively very low as compared with that of unheated peat or muck soil upon which *Pyronema* will not grow. Again the disappearance of the favorable condition for *Pyronema* from the soil seems to be considerably more rapid than the loss of concentration. At least, it is not to be expected that the food supply for the fungus is so rapidly diminished as to materially check the fungus growth after a period of only a few days. This statment needs of course, to be supported by the observation that more than one "crop" of fungus growth can occur upon any one heated soil.

That soils sterilized by heat at low temperatures or by antiseptics become a favorable medium for subsequent bacterial development has been shown repeatedly by various investigators beginning with the work of Hiltner and Störmer (25). The principal point of argument in recent years as to the effect of sterilization on the soil has been centered around the explanation of the cause of this increased bacterial activity. On account of the seemingly close correlation between the rate of bacterial development and of fungus growth on soils heated to different temperatures there apparently is no reason for distinguishing between the cause of the rapid development of these closely related microörganisms on sterilized soils. If this be true, then Russell's protozoan theory (64) would necessarily have to extend the activity of the phagocytic organisms to the limitation of fungus growth as well as to the limitation of bacterial development. The special favorableness which *Pyronema* finds for growth upon heated soils over that of other fungi is, however, apparently a matter for discussion aside from that of ordinary increased development of microörganisms in heated soils. The arguments of Seaver and Clark are apparently not concerned with this fact, but, using *Pyronema*

as an example, they attempt to explain why fungi in general find the soil a more favorable medium for growth. On the same assumption as previously made their conclusions might then be made to apply also to the explanation of increased bacterial activity in soil. The subject then finally resolves itself into two separate problems. (a) Why do microörganisms in general find a sterilized soil a more favorable medium for development? (b) Why does *Pyronema confluens* in particular find heated soils a more favorable medium for growth than do other fungi? The first problem will be dismissed with the simple statement that the writer's observations on a wide variety and type of heat sterilized soils have led to the opinion that in practically all cases the increased activity of microörganisms in sterilized soil can be explained on the basis of reduced competition (a wide variety of organisms are known to be negatively "chemotropic" to each other) in the presence of increased food supply without any special reference to the concentration of this food supply as such.

Seaver and Clark (68) assume as one basis for their theory on the growth of *Pyronema* in heated soil, that a concentration of soil solution favorable to fungi is unfavorable to green plants. The experiments of Raulin (57), and especially Bauman (3) who noted the relation of zinc salts to plants, and found that fungi are able to grow in solutions of zinc exceedingly toxic to higher plants, and Heald (23) who found the toxicity of carbolic and other acids to green plants greater than to fungi, illustrate the fact that certain fungi are relatively resistant to toxic substances. It is now generally recognized that the resistance of fungi to toxic agents is greater than that of green plants. It is not surprising, therefore, that fungi should grow on heated soils highly toxic to green plants regardless of the concentration of the soil solution, providing that these fungi find the substratum congenial because of reduced competition and increased food supply of either a general or a special nature. If we now reason a step further we should expect to discover that some fungi will find heated soils more favorable for growth than others, due to a modified food supply, as shown by Nikitinsky (45) and others, in the same way that certain green plants find heated soils more favorable for growth than others do since fungi as well as green plants differ in their food requirements. The fact remains, however that *Pyronema* is capable of reducing the toxicity to seed germination (and probably also to plant growth,) while growing on heated soil and at the same time of increasing the ammonia content of the soil. What relation the compound favorable to the growth of *Pyronema* has to the substance toxic to seed germination must, however, still remain obscure. The presumption is all in favor of it being a nitrogenous compound, but no good evidence exists on this point.

DISCUSSION OF RESULTS

Some of the conclusions drawn from the data presented in this paper have already been published in a preliminary paper (28). More data to support

the conclusions drawn have been secured since the publication of this first paper. On the other hand, some contradictory evidence on one point in particular has been obtained. That the instability of ammonium carbonates in heated soils, except when kept in a dry and unaerated condition, accounts for the gradual decrease of the injurious action is not supported by all the facts, since destruction of the carbonate was attributed to purely chemical or physical phenomena. The results secured since have shown the importance of microörganisms in the loss of toxicity. That the reduced toxicity is due to biological action upon ammonium carbonate, resulting in loss of gaseous ammonia, or to its fixation in the soil seems now most probable.

It seems evident from these studies that a large number of difficulties present themselves in the way of obtaining direct evidence upon the toxic action of the ammonia produced in heated soils. It has been felt in the course of the work that sufficient emphasis has not been placed upon quantitative determinations of the ammonia present, as such, for comparison with the toxicity of similar amounts in and out of the soil. On the other hand such evidence has seemed of minor value in view of the extraordinary influence of various chemical, biological, and physical processes in the soil on the ammonia relations. The extensive literature upon ammonia relations in soil abounds with evidence of the great variability of results secured in fertilization, ammonification, fixation, and absorption experiments. It seems worth while, however, to go to the literature to find confirmatory evidence for some of the conclusions presented in this paper.

With reference to toxicity of ammonia in soils to plant growth under conditions where it is reasonable to assume the amounts present are no greater than those produced in heated soils, mention may be made of the observations and experiments of Pitsch (54), Wagner (76), and Ehrenberg (14). Other investigators who have dealt with the susceptibility of seeds and plants to injury from ammonia in soils are Sigmund (69), Coupin (9), Ehrenberg (15), and Bokorny (4). Especially interesting in this connection are some of the results secured by Sigmund (69) and Ehrenberg (15) on the influence of zinc on soil. It has been shown that zinc is able to liberate ammonia from ammonium salts, which is then said to act corrosively on the plant roots through its hydroxyl ion, but on account of its easy dissociation, the ammonia partly volatilizes. Sterilization of the soil increases the injury while the action of the nitrate-forming organisms in removing ammonia compounds results in lessened or prevented injury. It appears, therefore, that we have in the action of zinc on soil a condition similar in many respects to that of heated soils.

As early as 1880, a very fundamental study was made by Nivet (46) on the relation of ammonia in the soil. This investigator found that ammonia occurs normally in soils as ammonium carbonate, and that the addition of ammonia in form of sulfate, results in ammonium carbonate being produced through its reaction with the calcium carbonate. The ammonium carbonate

being more or less unstable, considerable loss of ammonia from the soil may result, which loss varies greatly, however, with the nature of the soil. A great variation in the absorptive property of the soil was also noted in this connection, it being found that the ammonia given off from sandy soil was seven times as great as that from "humus" soil. In the presence of a considerable excess of pure carbon dioxide, the loss of ammonia by volatilization was reduced to an inappreciable amount. From the results of the investigations of Nivet (46) and others, it is evident that a consideration of the toxicity of ammonia in soils involves to a considerable extent its relations to the absorptive capacity present. The phenomena of absorption in soils has been known for nearly a century and many of the early workers as Way (77) had a good conception of the part played by this factor in heated soils, but more fundamental evidence from our standpoint has been brought together by recent workers, especially Richter (58), Wagner (76), Stoklasa (72), Prescott (56), and Cook (8). The most important facts advanced by these writers are as follows. Soils have a remarkable degree of power for absorbing gases, and especially ammonia, but they differ very widely in this capacity. In general the heavy types of soil, or those high in humus or vegetable matter, have a high absorptive capacity, and those of a light nature or low in vegetable matter have a low absorptive capacity. A striking variation from this rule, however, may be expected, and the actual or relative absorption of any soil can only be determined by actual trial. Absorption of ammonia is not, however, a simple physical process, but chemical and colloidal properties are also involved, together with many exterior influences, such as the temperature and moisture content of the soil. Chemical absorption may, for instance, result in the formation of an insoluble chemical compound. Colloidal matter increases the power of absorption. Calcium, especially calcium oxide, increases the absorptive power for ammonia in a decided way. Heating the soil decreases the absorption or fixation, a fact probably due largely to the action on humus and colloidal matter. High temperatures are generally unfavorable and low temperatures favorable to absorption, while increased moisture supply tends to permit of greater absorption.

With these facts in mind, it may be assumed that the following approximates the conditions in soil heated to 250°C. The maximum ammonia content is present in connection with a reduction in absorptive capacity of the soil. The amount of the measurable toxicity is then the result of the balance between the total ammonia produced on heating and the total absorptive capacity which exists in the heated soil. Heated soils with high ammonia content but also with high absorptive capacity, as is true in the case of peat, are therefore, less toxic than Waukesha silt loam with lower ammonia content, but with much lower absorptive capacity. Theoretically, a correlation should exist between the ammonia content and the absorptive capacity on the one hand and the toxicity to seed germination and plant growth on the other. The presence of numerous other disturbing factors renders such an

expectation practically unobtainable. The studies of Kanda (29) on the stimulatory action of metallic salts upon plant growth are merely cited to show the importance of such disturbing factors. In the case of copper sulfate, for instance, Kanda found it was difficult to get constant results in experiments because of the influence of such factors as the action of the salt upon the humus compounds of the soil, the influence of the time of the year, and especially temperature and moisture changes in the atmosphere. It was found that in a cold, moist atmosphere, the toxic action was much less than in a warm, dry atmosphere. Other meteorological and climatological factors were also found to influence results. In a similar manner, the influence of environmental factors, some of which are as yet not clearly understood, appear to complicate the data secured on seed germination and plant growth on heated soils.

The action of ammonia or ammonium compounds as plant food has been demonstrated (26). Lyon and Bizzell (42), Kelley and McGeorge (30) and others, as well as results of the author have also shown that nitrates are reduced in heated soils and that they are practically non-existent on soil heated to 250°C. Furthermore, according to some writers, heated soils become unfavorable for bacterial activity and although this conclusion is doubtful in all cases, it is at least probable that it takes some time before nitrification can reestablish itself to a decided extent. In the absence of nitrates, but with large supplies of ammonia present, it is probable that ammonia is being used directly by the plant as a source of nitrogen where plant growth occurs. That cereals in the presence of ammonia at least could develop in soils free from nitrates was concluded by Pitsch (54) as early as 1887. The researches of Kossowitsch (36) and Hutchinson and Miller (26) have added sufficient evidence to make it appear unquestionable that agricultural plants of various kinds can produce normal growth when supplied with nitrogen as ammonia in the absence of nitrates.

There is apparently room for difference of opinion as to the form of ammonia existing in heated soils. Ammonia as such, probably is never present in normal soils. When ammonium salts are added to soils, it is generally believed that they pass through several stages of decomposition and nitrification before being taken up by the plants. It is known that under ordinary conditions much ammonia exists in the soil as ammonium carbonate. On the other hand, it also appears that some of the ammonia enters into very stable combinations, from which it is no more readily separable (61). Eliminating for the present the biological factor in the soil, such as may be expected to occur in soils while heating or in highly heated soils stored dry, one is confronted with a chemical or at most a physico-chemical problem. There appears to be several reasons for the assumption that much of the ammonia produced in heated soils exists as some form of carbonate of ammonia. These are briefly: (a) The high production of carbon dixoide coincident with that of ammonia production may be expected to result in the combination of the two when cool-

ing to lower temperatures. (b) The stability of the high ammonia content in the soil when kept dry in the presence of reduced absorptive capacity argues against its existence as a gas. (c) The comparative ease with which ammonia is driven off from a soil extract at low temperatures indicates that it exists as an unstable compound. (d) The slight increase of acidity in heated soils indicates an excess of an acid radical, most probably carbon dioxide, which is produced in large amounts on heating, and hence reduces the possibility of ammonia remaining in the soil for any length of time as a free base.

When heated soils are kept moist and aerated, the carbonate of ammonia present is no doubt gradually dissociated into ammonia and carbon dioxide, only to be reabsorbed by the soil, changed or fixed into other forms by biological or other action, or possibly lost in small amounts to the atmosphere, depending upon the favorableness of the soil for the activities concerned. These changes in heated soils together with the ammonia removed by growing plants, where present, explain the loss of ammonia from heated soils and hence may account for reduced toxicity. As a matter of fact, however, an increase in ammonia actually occurs for a period of time following sterilization of the soil in spite of gradually reduced toxicity. Here the assumption must be made that the ammonia exists at this critical stage, not as toxic gas, but in various delicate stages of transition between humus compounds and ammonia on the one hand and between ammonia and nitrates on the other, and that these compounds are reduced to ammonia in the determinations of total ammonia present in the soil. In this process of transition, microörganisms play the main rôle, and we have shown that microörganisms are largely responsible for the destruction of the toxic property in the soil. It is of course equally probable that certain organic compounds, which may or may not be transition products of ammonia, and which result from decomposition of humus on heating, may be the toxic property involved. In contradiction of this we have only the evidence of the "qualitative" similarity of the action of ammonia and its compounds on seed germination as compared with that of highly toxic heated soils or the extracts of such heated soils. This evidence, together with the fact that ammonia or ammonium salts added to soils in correct amounts may produce marked similarity in action to that produced by heated soil on seed germination and plant growth, constitutes the main evidence for the conclusion that ammonia plays a large rôle in both the toxic and beneficial action of heated soils to seed germination and plant growth, although it may not be the only factor concerned. That all seeds and plants do not respond in a similar manner to heated soils is believed to be due to the difference in the selective permeability of the seed coat or of the plasma membrane to the toxic agents. This may also explain the ability of certain plants to assimilate nitrogen in the form of ammonia or ammonium salts either in the absence of, or in a reduced supply of other forms of nitrogen and hence may account in part for the beneficial action of heated soils on certain plants while they appear at the same time to be injurious to other species of plants.

SUMMARY

1. The purpose of this investigation has been primarily to arrive at some conclusions as to the nature of the action of sterilized soils upon plant growth, in order that their use in soil biological and phytopathological research as well as in prophylactic measures may be more clearly understood and more productive of reliable results.

2. Practically all soils when sterilized in the ordinary manner by heat at temperatures approximating 100-115°C. produce temporary retardation to seed germination and plant growth followed by increased rate of growth. The extent of this action varies very greatly with the soil, seed, and plants used and with the environmental conditions existing. To study the factors concerned, 7 different soils were subjected to widely varying treatments and conditions, and their action on various seeds and plants determined. Although some of the results secured are of a corroboratory nature, these have been extended or limited in their application.

3. On heating a soil to different temperatures, it was usually found that a gradual increase in toxicity occurs to seed germination and to early plant growth which reaches its maximum at approximately 250°C., but gradually decreases to practically no toxicity on soils heated to 350°C. or above.

4. The time required for recovery from this toxic action is usually directly proportional to the intensity of toxicity produced, but the final beneficial action is often greatest on the soils exhibiting the greatest injurious action on early plant growth.

5. Different soils vary markedly in their behavior upon heating to the same temperatures both in toxicity and in beneficial action to seeds and plants, and this is not seemingly correlated with any single distinguishing character in the soil, but is correlated rather with the balance of all the factors concerned.

6. Seeds vary greatly in their sensitiveness to the toxic action. Lettuce and clover seed are for instance, very susceptible to the toxic substance, whereas seeds of wheat, buckwheat or flax are very resistant. The degree of sensitiveness of seeds is roughly characteristic of their genetic relationship. The Gramineae and the Cucubitaceae are usually resistant and the Leguminoseae and Solanaceae are, as far as determined, more susceptible.

7. With the seeds resistant to the toxic action marked acceleration of rate of germination may occur on even highly heated soils. This is no doubt another expression of the same substances that cause retardation in more susceptible seeds. Seeds classed as susceptible may on the other hand show accelerated germination on soils not productive of high toxicity on heating or upon heating soils to low temperatures only.

8. Growing plants differ markedly in their sensitiveness to the action of heated soils. Soils very toxic to certain plants, such as tomatoes, may be very beneficial to others such as wheat. The similarity of the behavior of growing

plants and germinating seeds in this respect suggests that the injurious and beneficial substance in both cases may be the same. Toxicity to seed germination, however, is seemingly not always correlated with toxicity to plant growth and vice versa. Furthermore, sensitiveness of seeds to the toxic agent in germination is not indicative of the behavior of the same species in its growth on the same soil.

9. The growth of fungi on heated soil is correlated with the toxicity to seed germination and plant growth on any one soil. The growth of *Pyronema* especially has been studied. This and other fungi and apparently bacteria also grow best in soil heated to 250°C., diminishing in rate and profuseness of growth at lower or higher temperatures of heating.

10. The ammonia content of a soil heated to different temperatures is highest on heating to about 250°C. and diminishes gradually at higher and lower temperatures of heating. This is also true for the concentration of the soil solution. Ammonia content and concentration of the soil solution are therefore roughly correlated with the degree of toxicity to seed germination and early plant growth and the extent of the beneficial action to late growth of green plants and to growth of lower microörganisms in any one soil.

11. There is apparently, however, no correlation between these factors when different soils are compared to each other. The toxicity of heated Waukesha silt loam with a relatively low ammonia content is, for instance, much greater than that of heated peat with a relatively much higher ammonia content. The absorptive capacity of the two soils is, however, vastly different, and this in turn markedly influences the action of toxic compounds produced in the soil. In water extracts of heated soils, the toxicity to seed germination is more directly proportional to the ammonia content. It seems that the toxicity of the soil is, therefore, not only determined by the amount of the toxic agent produced by heating, but also by the absorptive capacity of the soil for the toxic agent as well as by a number of other factors, the additive and subtractive value of which gives a balance of toxicity very difficult to properly analyze.

12. The addition of ammonia as such to soil in varying amounts may be made to produce a condition similar in many respects to that produced by heated soils on seed germination and plant growth.

13. Very similar "qualitative" reactions can be produced with certain seeds on highly toxic heated soils or their extracts and with certain strengths of ammonia. These reactions do not seem to be reproducible with chemicals other than ammonia or ammonium salts.

14. It is believed that much of the toxic action in heated soils is due to the ammonia produced and that it exists in the heated dry soils largely as ammonium carbonate, which is, however, gradually decomposed under normal growing conditions.

15. All the toxic properties in heated soils are not, however, believed to be the same. Certain changes termed "chemical" injuries are seemingly due to quite different causes than ordinary retardation.

16. The toxic property is volatile and is destroyed or changed to non-toxic compounds in soils kept under normal growing conditions. This has been shown to be due to the activity of ordinary soil flora, which, however, may apparently at the same time increase the amount of ammonia present. *Pyronema confluens* has also been shown to be efficient in both these respects. The reduced toxicity in the presence of increased ammonia content in the soil is believed to be explained by its existence in various delicate transition stages rather than as true ammonia due to the activity of soil organisms and that these transition products are broken down when the ammonia determinations are made. The reduction of the toxic property to seed germination and plant growth by the activity of soil flora has been shown and is contrary to Pickering's conclusion that loss of toxicity in storage of heated soils is an oxidation process which may go on under aseptic conditions.

17. The beneficial action of highly heated soils is believed to be due in a considerable measure to the ammonia liberated on heating, since increased growth may result in almost total absence of nitrates or in heated soil under aseptic conditions in spite of a considerable toxic action upon the roots. The fact that certain green plants are able to take up their nitrogen in the form of ammonia is believed partially to explain the variation of sensitiveness of plants to heated soils.

18. The temperature of the soil is an important factor in determining the extent of the toxic and beneficial action to plant growth. The toxic action disappears more slowly and is more destructive at low soil temperatures (below about 25°C.) than at higher temperatures.

19. Observations on the growth of *Pyronema* seem to indicate that the favorableness of heated soils to its growth is not entirely one of concentration of soil solution, as argued by Seaver and Clark. *Pyronema* will grow on heated soils very low in concentration as compared with other unheated soils on which growth never occurs. All microörganisms appear to grow better on the soils of higher concentration of soil solution, owing to increased food supply, but the type of organism and the extent of its growth will vary with the competition at hand and the kind of food materials present. Fungi differ in their food requirements in much the same way as green plants, and heating the soil no doubt produces chemical substances especially favorable to the growth of *Pyronema*.

20. The conclusions drawn here are considered to apply particularly to highly heated soils, although it is not believed that any fundamental difference exists between ordinary steam sterilized soils and highly heated soils reinoculated with normal soil flora.

21. Although the injurious action of heated soils on plant growth has been brought into the foreground in this paper, it is not desired to leave the impression that heat sterilized soils are of questionable value in research and practice. The opposite is rather true and little hesitancy need be felt in recommending steam sterilization of soil for practical purposes or for use in research problems

where it is necessary to eliminate certain organisms from the soil. But, one must be prepared to expect a short period of retardation of growth followed by a beneficial action, and in special cases, with certain soils or plants or under certain environmental conditions, a very marked interference with the normal development of plants.

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PLATE 1

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. Showing the difference in response of different soils on heating to 110°C. on the growth of tomatoes. *A*, Check, soil not heated; *B*, heated soil. Pots 1A–1B, Miami silt loam; pots 2A–2B, muck; pots 3A–3B, red clay; pots 4A–4B, greenhouse compost; pots 5A–5B, peat; pots 6A–6B, virgin sandy loam.

Fig. 2. Effect of heating virgin sandy loam to different temperatures, showing the retarding influence on plant growth. *1*, Not heated; *2*, heated to 50°C.; *3*, 100°C.; *4*, 150°C.; *5*, 200°C.; *6*, 250°C.; *7*, 300°C.; *8*, 350°C.

Fig. 3. Growth of radish on muck soil heated to different temperatures illustrating the beneficial action to late plant growth. *A*, Not heated; *B*, heated to 115°C.; *C*, heated to 250°C.; *D*, heated to 350°C.

FIG. 1

FIG. 2

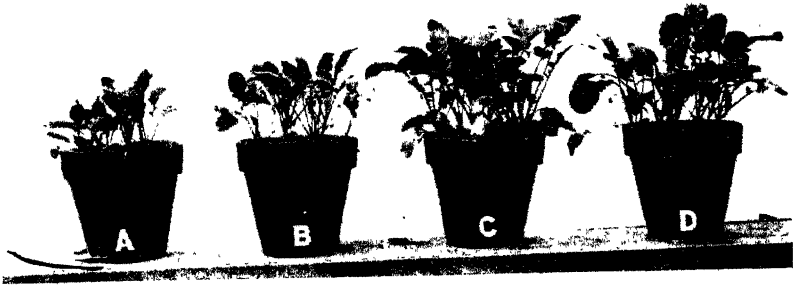

FIG. 3

PLATE 2

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. Growth of soy beans and tobacco on Waukesha silt loam soil heated to different temperatures, showing the beneficial action of the lower and higher temperatures of heating on soybeans, but the retarded recovery at 150°–250°, whereas the tobacco has recovered and shows beneficial action at these temperatures. *A*, Not heated; *B*, heated to 50°C.; *C*, 100°C.; *D*, 150°C.; *E*, 200°C.; *F*, 250°C.; *G*, 300°C.; *H*, 350°C.

Fig. 2. Showing the variation in behavior of different crops on virgin sandy loam soil heated to 115°C. *A*, Tomatoes on unheated soil; *B*, tomatoes on heated soil; *C*, wheat on unheated soil; *D*, wheat on heated soil; *E*, buckwheat on unheated soil; *F*, buckwheat on heated soil

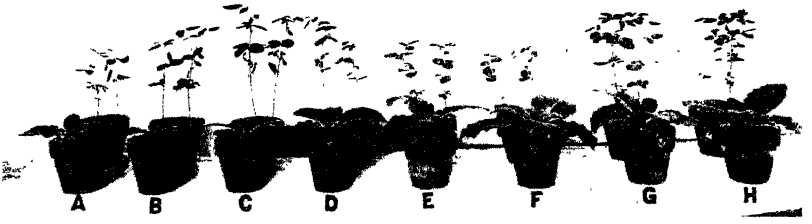

FIG. 1

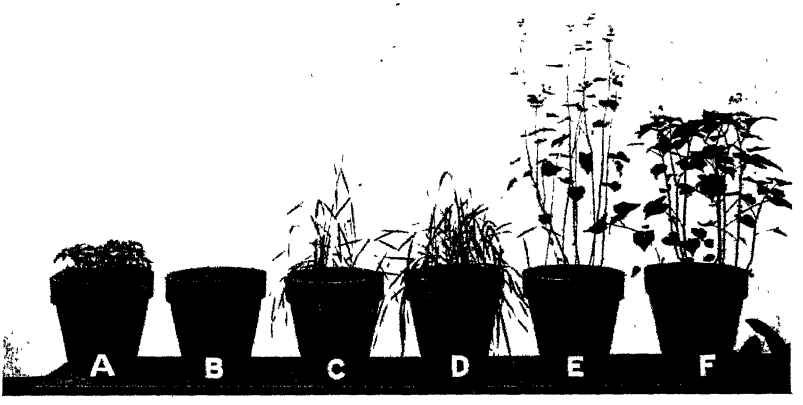

FIG. 2

PLATE 3

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. The growth of tomatoes on muck soil heated for different lengths of time at 115°C., showing an appreciable beneficial action at the longer times of heating. *1*, Not heated; *2* heated 10 minutes; *3*, 20 minutes; *4*, 40 minutes; *5*, 80 minutes; *6*, 160 minutes.

Fig. 2. The growth of tomatoes on virgin sandy loam heated for different lengths of time at 115°C. showing a decided injurious action at the longer times of heating. *A*, Not heated; *B*, heated 10 minutes; *C*, 20 minutes; *D*, 40 minutes; *E*, 80 minutes; *F*, 160 minutes.

FIG. 1

FIG. 2

PLATE 4

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. Showing the influence of soil temperature on the extent of the toxic action of heated Miami silt loam soil on the growth of tobacco. *1A*, Not heated and grown at 28–29°C.; *1B*, heated to 110°C. and grown at 28–29°C.; *2A*, not heated and grown at 23–24°C.; *2B*, heated to 110°C. and grown at 23–24°C.

Fig. 2. Showing the influence of soil temperature on the extent of the toxic action of virgin sandy loam on the growth of tomatoes. *1A*, Not sterilized soil and grown at 17–18°C.; *1B*, sterilized soil and grown at 17–18°C.; *2A*, not sterilized soil and grown at 23–24°C.; *2B*, sterilized soil and grown at 23–24°C.; *3A*, not sterilized soil and grown at 28–29°C.; *3B*, sterilized soil and grown at 28–29°C.

FIG. 1

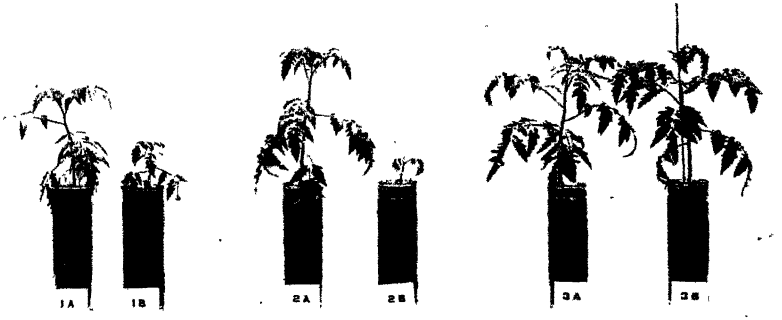

FIG. 2

PLATE 5

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. Illustrating the influence of soil temperature on the toxic action of heated soil on the roots of tomato plants. The higher soil temperatures greatly reduced the extent of the toxic action.

Fig. 2. "Chemical" injury of heated greenhouse compost (manure and sod mixture) on tomato plant. This injury occurred suddenly after plant had been growing normally for some time. *A*, Check, soil not heated; *B*, soil heated to 110°C.

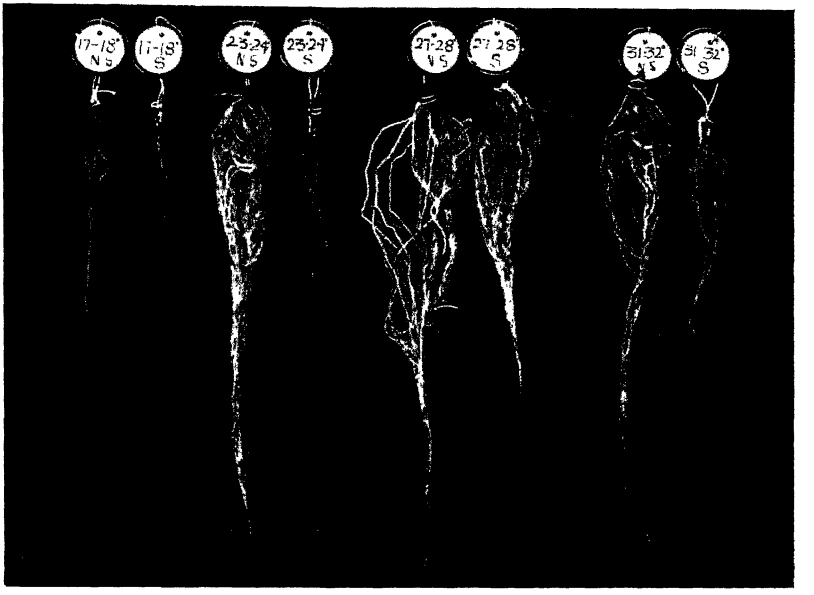

FIG. 1

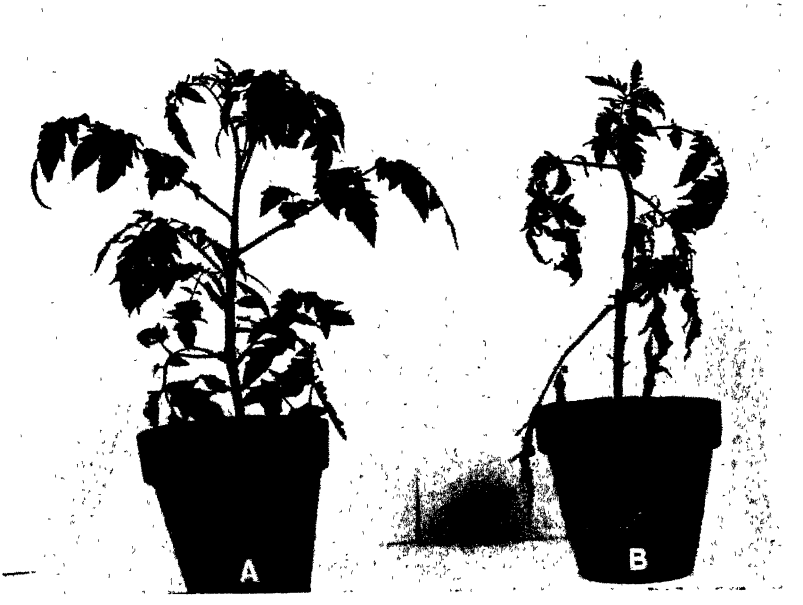

FIG. 2

PLATE 6

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

"Chemical" injuries to the foliage of various plants as a result of growing on heat sterilized soils. *A*, Mottled leaf of tomatoes quite common on certain heated soils; *B*, collapse of leaflets and petiole of tomato grown on heated soil; *C*, "leaf spots" of tobacco; *D*, marginal spotting on soybeans; *E*, injury to midribs and veins of leaflets of soybeans resulting in curling; *F*, "specking" of cowpeas.

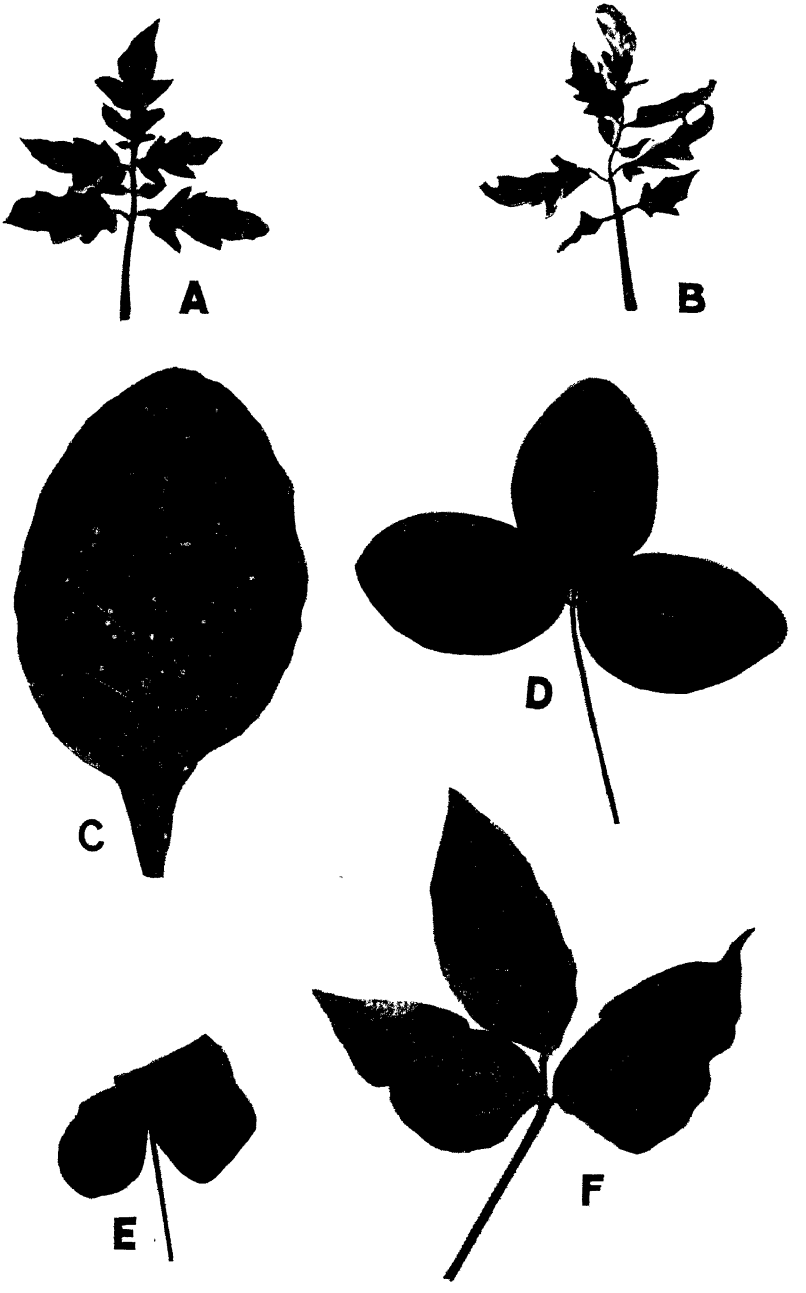
A
B
C
D
E
F

PLATE 7

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. The growth of *Pyronema* on soil heated to different temperatures. *A*, Not heated; *B*, heated to 50°C.; *C*, 100°C.; *D*, 150°C.; *E*, 200°C.; *F*, 250°C.; *G*, 300°C.; *H*, 350°C.

Fig. 2. Growth of tomatoes on silt loam soil heated to different temperatures, as compared with treatment with varying amounts of ammonia, as ammonium carbonate, illustrating the similarity in responses obtainable. *1A*, Not heated; *2A*, heated to 150°C.; *3A*; heated to 200°C.; *4A*, heated to 250°C.; *1B*, no treatment; *2B*, treated with 0.1 per cent ammonium carbonate; *3B*, treated with 0.2 per cent ammonium carbonate; *4B*, treated with 0.4 per cent ammonium carbonate.

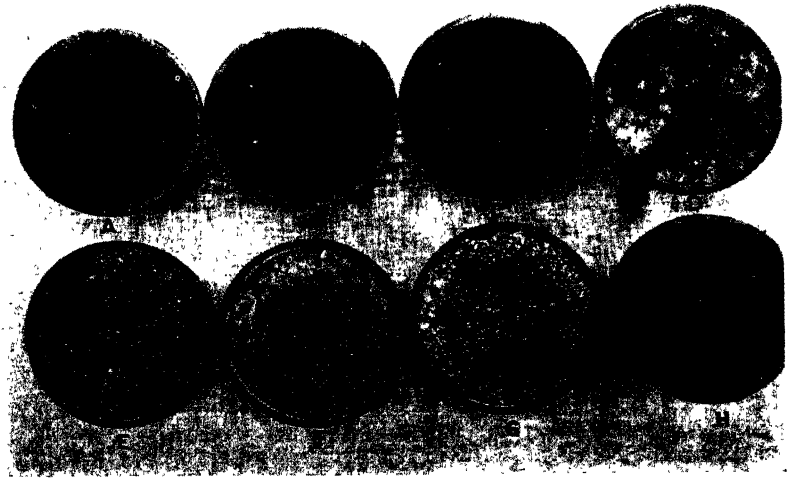

FIG. 1

FIG. 2

PLATE 8

THE INFLUENCE OF HEATED SOILS ON SEED GERMINATION AND PLANT GROWTH

Fig. 1. Growth of wheat on fine sandy loam under various conditions with respect to activity of microörganisms. *A*, Sterilized soil reinoculated with normal soil flora; *B*, setrilized soil under aseptic conditions; *C*, unsterilized soil check.

Fig. 2. Showing the relative development of tops and roots of wheat grown on heated and unheated soil under varying conditions with respect to activity of microörganisms. *A*, Sterilized soil reinoculated with normal soil flora; *B*, sterilized soil, and aseptic conditions; *C*, unsterilized soil check. Note especially the stunted root systems in *B*, but better aerial growth than in *C* in spite of the toxic action. The loss of the toxic action on roots in reinoculated soil is shown by comparing *A* with *B*.

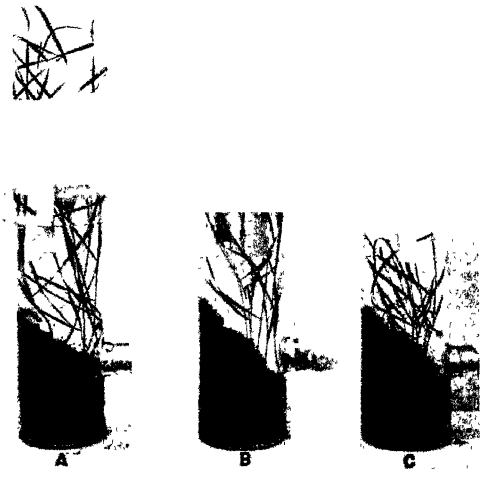

FIG. 1

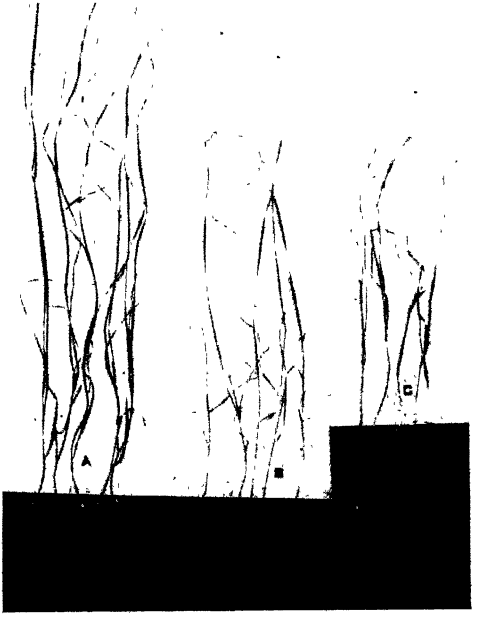

FIG 2

THE INHIBITION BY STABLE MANURE OF THE INJURIOUS EFFECTS OF ALKALI SALTS IN SOILS

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When one sums up all of the investigations, and there have been many, which have dealt with the problem of control of alkali in soils, he is impressed with the large degree of failure in which these efforts have resulted. It is not our purpose here to review the whole situation and to comment on the probable causes for the failures to which we have referred. We merely wish to call attention now to the following facts. There have been two methods of attack for the problem. The first has concerned itself with inhibiting the toxic effects of the salts without removing them from the soil. The second has attempted to eradicate the evil by the total removal of the alkali salts from the affected soil. We hope at another time and place to discuss in detail the second method of attack, its *raison d'etre*, and its results. The first method or class of methods has been the subject of considerable investigation in this laboratory and has received some discussion at our hands. This class of methods comprises a number of sub-classes which it is unnecessary now to mention. It suffices for our present purposes to state that one of them consists in the use of easily decayed organic matter on alkali soils. This method of controlling alkali has, it is reported, frequently been crowned with success for short periods, at least when used by some farmers. The latter did not, of course, concern themselves with the causes or reasons for such protective action on the part of organic matter, but insisted that when barnyard manure was added to alkali soils, which permitted of only sparse plant growth, it effected marked changes in the soil and made possible good plant growth thereon. For some reason farmers have not been encouraged by experiment stations in the use, as a remedial measure, of barnyard and stable manures on alkali lands. It appears that a general impression of its practical inutility as an ameliorant for alkali soil has been abroad. It occurred to us in 1912 that the insistent claims of some farmers on the efficacy of manure on alkali land deserved some attention. Moreover, there appeared to be some sound theoretical basis, as we shall show below, for believing such claims to be correct.

PLAN OF THE EXPERIMENT

The experiment was conducted in a greenhouse. The plant containers were paraffined 9-inch earthenware flower pots, each of which received about 5 kilogram of soil. The soil used was a class or type commonly referred to as Berkeley clay adobe. It was collected on the campus of the University of California. The soil and manure used were both sifted through a ¼-inch mesh sieve. The dry manure and dry soil were thoroughly mixed in all pots in which manure was used. The amounts of manure used varied as shown in the accompanying tables. Duplicate cultures were run for all treatments, and control untreated pots to which no manure and no salt had been added were included in the experiment as well as control pots receiving the alkali salts but no manure. The salts were added in solution in the following amounts prior to the first planting: NaCl 0.3 per cent, Na_2CO_3 0.3 per cent, Na_2SO_4 0.6 per cent. Prior to the second planting, as much salt again was added, based on the dry weight of the soil, thus doubling the percentages just given. Losses of Na_2SO_4 were sustained through "creeping" in spots in which the pots had not been thoroughly paraffined. In the case of the other salts, however, there was no difficulty in maintaining the original salt content throughout the experiment.

Four successive crops were grown in the same pots in the following seasons: the fall and winter of 1913, 1914, 1915, and 1916. In order to allow for the so-called plant food substances added in the manure and make them equivalent so far as possible in all cultures, pots 1, 2, 3, 4, 5, 6, 7, 8, 17, 18, 19, 20, and 21 received applications of NH_4NO_3 and NaH_2PO_4. Thus, all pots receiving manure equivalent to 20, 40, 60, and 80 tons per acre and the two kinds of control pots above mentioned were so treated. The seed used was from a choice lot of seed of a selected variety of Beldi barley grown on the University Farm at Davis. The soil moisture was kept at the optimum as nearly as possible throughout the growing season. In harvesting, the straw, grain, and root yields were determined in every case except as otherwise stated below. The manure employed was in a very dry condition and contained only 25 per cent of moisture. The results of the experiment are given in the accompanying tables. We shall discuss separately the results obtained with every salt throughout the four seasons.

Series 1. NaCL

We are fully cognizant of the considerable variability which characterizes the values obtained by us from duplicate cultures both treated and untreated. Further, we are aware of the significance of such variability to the validity of conclusions drawn from the experiment. But with all this recognized, a study of tables 1, 2, 3 and 4 shows emphatically that organic matter in soils exerts a profound effect on the salts present with it, or that it so affects plant

growth on the soil as to make it proof against the toxic effects of the salts. The former is, of course, the more likely, but the latter is at least possible. Whatever the interpretation may be, it seems quite clear that organic matter, in sufficient quantity, exerts a protective effect on plants growing on soil containing NaCl.

In the first crop, in which it will be remembered the soil received only half the amount of NaCl that it did prior to growing the following three crops, 0.3 per cent NaCl gave a marked depression in the yield of dry matter, as is

TABLE 1

NaCl *series*. *First crop*

NUMBER	NaCl ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.3	20	32.0	29.00			32.0	29.00	5.1	5.45	37.1	34.45
2	0.3	20	26.0				26.0		5.8		31.8	
3	0.3	40	44.1	45.25			44.1	45.25	8.2	6.75	52.3	52.00
4	0.3	40	46.4				46.4		5.3		51.7	
5	0.3	60	46.3	48.65			46.3	48.65	10.0	9.10	56.3	57.75
6	0.3	60	51.0				51.0		8.2		59.2	
7	0.3	80	45.0	53.70			45.0	53.70	6.2	8.10	51.2	61.80
8	0.3	80	62.4				62.4		10.0		72.4	
9	0.2	100	58.0	54.00			58.0	54.00	8.2	9.50	66.2	63.50
10	0.3	100	50.0				50.0		10.8		60.8	
11	0.3	120	66.0	55.50			66.0	55.50	6.0	7.10	72.0	62.60
12	0.3	120	45.0				45.0		8.2		53.2	
13	0.3	140	44.6	59.70			44.6	59.70	6.8	8.65	51.4	68.35
14	0.3	140	74.8				74.8		10.5		85.3	
15	0.3	160	55.0	61.00			55.0	61.00	8.0	7.00	63.0	68.00
16	0.3	160	67.0				67.0		6.0		73.0	
17	0.3		23.8	21.15			23.8	21.15	2.3	3.40	26.1	24.55
18	0.3		18.5				18.5		4.5		23.0	
19			47.0	42.50			47.0	42.50	6.7		53.7	50.47
20			40.0				40.0		11.7	7.97	51.7	
21			40.5				40.5		5.5		46.0	

indicated by a comparison of the yields of the untreated pots with those of the NaCl treated ones. When, however, to the soil containing 0.3 per cent NaCl manure is added at the rate of 20 tons per acre by weight, our data show that it inhibits the toxic effect of NaCl to a considerable degree. Moreover, when manure is added at the rate of 40 tons per acre or more, the toxic effect of 0.3 per cent NaCl is entirely obliterated and the cultures behave as if no salt were present in the soil. Indeed the larger applications of manure actually seem to add to their inhibitory effect a stimulating effect. With

regard to the latter point, it is of course difficult again to say whether the manure acts to prevent the toxicity of the NaCl or whether its stimulating effect on the plant is so great as to mask the toxic properties of the salt, thus yielding in the final results merely the algebraic sum of the stimulating effect of the manure and the depressing effect of the NaCl. However that may be, the net result of the manure applications, under the conditions studied, was to prevent partly or wholly, depending on the quantity used, the depressing effect on barley growth of the NaCl. As is usual under conditions of very

TABLE 2

NaCl *Series. Second crop*

NUMBER	NaCl ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	10.00	9.23	7.00	6.77	17.0	16.00	2.20	2.10	19.20	18.10
2	0.6	20	8.45		6.55		15.0		2.00		17.00	
3	0.6	40	10.10	10.85	6.80	6.70	16.9	17.55	2.50	3.10	19.40	20.65
4	0.6	40	11.60		6.60		18.2		3.70		21.90	
5	0.6	60	21.80	21.96	13.20	8.79	35.0	30.75	2.90	3.40	37.90	34.15
6	0.6	60	22.12		4.38		26.5		3.90		30.40	
7	0.6	80	30.98	31.34	11.02	10.36	42.0	41.70	2.30	2.95	44.30	44.65
8	0.6	80	31.70		9.70		41.4		3.60		45.00	
9	0.6	100	45.70	46.31	11.80	11.09	57.5	57.40	2.00	3.20	50.50	60.60
10	0.6	100	46.92		10.38		57.3		4.40		61.70	
11	0.6	120	40.33	36.94	19.67	15.56	60.0	52.50	2.30	2.60	62.30	55.10
12	0.6	120	33.55		11.45		45.0		2.90		47.90	
13	0.6	140	40.05	40.71	20.15	18.89	60.2	59.60	2.75	2.58	62.95	62.18
14	0.6	140	41.37		17.63		59.0		2.40		61.40	
15	0.6	160	51.40	48.87	13.60	17.13	65.0	66.00	2.75	2.60	67.75	68.60
16	0.6	160	46.35		20.65		67.0		2.45		69.45	
17	0.6		16.90	10.20	1.10	3.05	18.0	13.25	1.98	1.24	19.98	14.49
18	0.6		3.50		5.00		8.5		0.50		9.00	
19			19.30		4.40		23.7	31.60	7.7	8.10	31.40	39.70
20			37.20		2.30		39.5		8.5		48.00	

heavy vegetative growth in our greenhouse, no grain was produced in the first crop. The root development followed, in general, the development of the tops, as the figures for the dry weight of roots indicate. Other points of interest in the first crop are common to all the series studied and will be discussed below.

In the second crop the doubling of the quantity of salt seemed to result in no greater depression in barley yield than that induced by 0.3 per cent NaCl. This is difficult to explain. On the other hand, the yield on the untreated

control pots decreased considerably; and the protective effect of manure applications at the rate of 20 and 40 tons per acre is apparent. This point is, however, very difficult of determination owing to the markedly discrepant values obtained from the duplicate cultures receiving only the 0.6 per cent NaCl without any manure additions. While, however, the question of the 20 and 40 ton applications of manure and their effects on the second crop is in serious doubt, there can be no question that the larger applications of manure are extremely effective, as they were in the first crop. When we

TABLE 3

NaCl *series. Third crop*

NUMBER	NaCl ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	11.40	11.40	1.80	2.60	13.20	14.00	1.32	1.51	14.52	15.51
2	0.6	20	11.40		3.40		14.80		1.70		16.50	
3	0.6	40	14.10	14.10	4.10	4.20	18.20	18.30	1.04	1.15	19.24	19.45
4	0.6	40	14.10		4.30		18.40		1.26		19.66	
5	0.6	60	19.20	18.90	3.40	4.80	22.60	23.70	1.45	1.45	24.05	25.15
6	0.6	60	18.60		6.20		24.80		1.45		26.25	
7	0.6	80	21.75	20.60	7.25	6.70	29.00	27.30	1.20	1.38	30.20	28.68
8	0.6	80	19.45		6.15		25.60		1.55		27.15	
9	0.6	100	28.15	25.92	6.85	6.58	35.00	32.50	2.00	2.25	37.00	34.75
10	0.6	100	23.70		6.30		30.00		2.50		32.50	
11	0.6	120	41.10	39.10	12.30	9.40	53.40	48.50	2.24	1.87	55.64	50.37
12	0.6	120	37.10		6.50		43.60		1.50		45.10	
13	0.6	140	46.00	39.50	6.00	6.00	47.00	42.50	1.00	0.95	48.00	43.45
14	0.6	140	38.00		6.00		38.00		0.90		38.00	
15	0.6	160	26.25	33.02	3.75	6.28	30.00	39.30	1.30	1.67	31.30	40.97
16	0.6	160	39.80		8.80		48.60		2.04		50.64	
17	0.6		7.90	8.30	0.30	1.00	8.20	9.30	0.34	0.27	8.54	9.57
18	0.6		8.70		1.70		10.40		0.20		10.60	
19			8.05	11.95	2.15	1.45	10.20	13.40	1.95	2.48	12.15	15.88
20			12.80		1.20		14.00		3.26		17.26	
21			15.00		1.00		16.00		2.24		18.24	

consider the grain production alone and as separate from the straw yields, there seems to be no question that even the smaller manure applications are effective in preventing the toxic effects of the NaCl in the second crop. It should also be noted that with the increase in size of the manure application, its protective effect increases up to and including the 140 ton manure application. This is more true for grain than for straw production. Root production is depressed throughout in the second crop by the NaCl application, as is strikingly seen by comparing the root yields of the untreated pots with

those of all other pots in the series. Nevertheless, here again we find a definitely protective effect of the manure, as the figures indicate. But it should be noted that there is lacking the progressive increase in root production with increases in the size of the manure applications which characterizes the grain yields particularly, but to some extent also the straw yields in the same series.

In the third crop, the characteristic discrepancies between duplicate cultures are again in evidence. On the whole, however, the concordance between them is good, and it may be safe to assume from the data that evidence is at

TABLE 4

NaCl *series. Fourth crop*

NUMBER	NaCl ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	10.50	10.50	4.50	4.65	15.00	15.15	4.00	3.95	19.00	19.10
2	0.6	20	10.50		4.80		15.30		3.90		19.20	
3	0.6	40	10.40	9.15	4.00	4.05	14.40	13.20	3.00	2.25	17.40	15.45
4	0.6	40	7.90		4.10		12.00		1.50		13.50	
5	0.6	60	8.00	10.43	2.00	3.82	10.00	14.25	2.70	3.35	12.70	17.60
6	0.6	60	12.85		5.65		18.50		4.00		22.50	
7	0.6	80	20.80	22.70	12.10	11.10	32.90	33.80	2.50	3.75	35.40	37.55
8	0.6	80	24.50		10.10		34.70		5.00		39.70	
9	0.6	100		18.10		10.60		28.70		4.00		32.70
10	0.6	100	18.10		10.60		28.70		4.00		32.70	
11	0.6	120	19.30	22.70	10.20	13.30	29.50	36.00	7.00	6.90	36.50	42.90
12	0.6	120	26.10		16.40		42.50		6.80		49.30	
13	0.6	140	30.00	32.00	15.00	14.50	45.00	46.50	6.50	6.75	59.50	53.25
14	0.6	140	34.00		14.00		48.00		7.00		55.00	
15	0.6	160	29.50	28.65	14.50	18.45	44.00	47.10	6.20	5.90	50.20	53.00
16	0.6	150	27.80		22.40		50.20		5.60		55.80	
17	0.6		6.90	7.15	2.20	2.20	9.10	9.35	1.30	1.35	10.40	10.70
18	0.6		7.40		2.20		9.60		1.40		11.00	
19			7.30	7.80	2.70	3.73	10.00	11.53	4.50	5.17	14.50	16.70
20			8.90		4.20		13.10		7.00		20.10	
21			7.20		4.30		11.50		4.00		15.50	

hand of the definite depression in barley growth produced by 0.6 per cent NaCl in this crop. The protective effect of the manure is even more certainly shown, and the evidence for it is in this instance certainly beyond question. As was true in the case of the second crop, there is in the third crop the progressively increasing protective effect with the increase in the quantity of manure applied up to and including the cultures receiving the 120 ton application. Applications greater than that seem to be without effect in increasing the yield. While thus we have evidence of the increase in its effects at

least up to a certain amount accompanying the increase in the amount of manure employed, it is also clear that even the smallest application used was distinctly effective. The marked protective effect noted, while applying in general to the dry matter production is, however, more true of the grain yields than of the straw and root yields.

In the fourth crop, as in the second, there is insufficient evidence on which to claim the depressing effect on straw production in the clay adobe soil of 0.6 per cent NaCl, but there seems to be no lack of evidence of such effect on the grain and root yields. As was true in the third crop, there can scarcely be any doubt that manure applications of all magnitudes exert inhibiting effects to the action of NaCl. Again the effects are much smaller with the three smaller manure applications but they are very marked with applications of 80 tons or more per acre, and increase from that through the higher applications reaching a maximum at the 140 ton applications. The grain yields and straw yields seem to be improving from crop to crop, and this seems to be true particularly from the third to the fourth crop.

Series 2. Na_2SO_4

Tables 5, 6, 7 and 8 give the results obtained with the 4 successive crops of barley in the Na_2SO_4 series. The amount of Na_2SO_4 used with the first crop (0.6 per cent) was evidently not great enough on the clay adobe soil to give any indisputable depression to the growth of barley. This is particularly true for straw production alone in the figures, from which in table 5 there is no evidence that the salt application definitely depressed the yield below that in the untreated control pots. In the root yields of the same crop, however, the evidence seems pretty reliable as a guide to the conclusion that 0.6 per cent Na_2SO_4 does definitely depress the root development of the barley plant on the clay adobe soil. No grain having been produced in the first crop of the Na_2SO_4 series, as was the case in the corresponding crop of the NaCl series, there is little cause for further comment on table 5. It is interesting to note, however, that while there were some differences between their effects, the different manure applications did not seem to make corresponding differences in the yields obtained. Another point is worthy of note here and that is that so large an amount of Na_2SO_4 as 0.6 per cent should be so slightly effective (roots) and so ineffective (straw) in regard to depressing plant development. It is very clear that in the soil with which we are dealing there is a factor of a strikingly inhibiting character to the effects of Na_2SO_4 on the barley plant. This factor must be the large internal surface offered by the clay adobe soil by means of which the salt is so largely adsorbed. It can scarcely be accounted for in any other way but it is not improbable that antagonism plays a large part here. It cannot be the resistance of the barley plant, since the latter from the same strain of seed when grown on a light soil is profoundly depressed by much smaller quantities of Na_2SO_4.

With the doubling of the quantity of Na_2SO_4 preceding the second crop, we note in table 6 what seems to be a very definite depression in yield due to the salt, despite the great discrepancy between the yields of the duplicate untreated control pots. The justification for the latter qualification may be found in the figures for the yields obtained in the pots receiving the smaller manure applications. All these statements apply fairly to the straw yields and very definitely to the root yields, but not to the grain yields. In the latter, we not only see no depression due to the effects of the Na_2SO_4 but there

TABLE 5

Na_2SO_4 *series. First crop*

NUMBER	Na_2SO_4 ADDED TO SOIL	MANURE RATE PER ACRE, SURFACE AREA	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	ANERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	31.5	35.75			31.5	35.75	5.8	5.40	37.3	41.15
2	0.6	20	40.0				40.0		5.0		45.0	
3	0.6	40	48.5	46.75			48.5	46.75	8.0	9.00	56.5	55.75
4	0.6	40	45.0				45.0		10.0		55.0	
5	0.6	60	57.0	54.50			57.0	54.50	8.5	8.50	65.5	63.00
6	0.6	60	52.0				52.0		8.5		60.5	
7	0.6	80	52.5	52.10			52.5	52.10	6.0	7.75	58.5	59.85
8	0.6	80	51.7				51.7		9.5		61.2	
9	0.6	100	53.2	53.30			53.2	53.30	10.2	9.85	63.4	63.15
10	0.6	100	53.4				53.4		9.5		62.9	
11	0.6	120	53.4	54.70			53.4	54.70	6.0	6.35	59.4	61.05
12	0.6	120	56.0				56.0		6.7		62.7	
13	0.6	140	50.5	52.75			50.5	52.75	10.0	8.25	60.5	61.00
14	0.6	140	55.0				55.0		6.5		61.5	
15	0.6	160	45.5	44.65			45.5	45.15	7.5	6.65	53.0	51.80
16	0.6	160	44.8				44.8		5.8		50.6	
17	0.6		37.5	39.50			37.5	39.50	4.6	4.25	42.1	43.75
18	0.6		41.5				41.5		3.9		45.4	
19			47.0	42.50			47.0	42.50	6.7	7.97	53.7	50.47
20			40.0				40.0		11.7		51.7	
21			40.50				40.5		5.5		46.0	

seems actually to have been some stimulation to increased yield over the grain yield of the untreated control pots. However, the manure applications, beginning with 60 tons per acre and going up, are clearly very effective in preventing injury by the Na_2SO_4 both as regards straw and grain yields, and probably also distinctly so for the root yields. In general, therefore, we feel justified in concluding from the data in table 6 that 1.2 per cent Na_2SO_4 in the Berkeley clay adobe soil is distinctly injurious to the development of the barley plant and that its injury may be lessened or entirely inhibited by considerable manure applications.

In the third crop, as represented by table 7, we find the most definite and interesting data of the Na_2SO_4 series. They seem to show beyond a peradventure that in that stage of its development the soil is no longer able to mask the toxic effects of the Na_2SO_4 as it appeared to be able to do at least in some measure in the preceding crop. They also show, as regards straw production, that even the smallest manure application employed is effective in overcoming the injurious effects of the Na_2SO_4 as used, that 40 tons of manure per acre is no more effective in that direction than 20 tons, that the

TABLE 6

Na_2SO_4 *series. Second crop*

NUMBER	Na_2SO_4 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.2	20	19.50	16.55	9.50	8.95	29.0	25.50	2.80	2.05	31.80	27.65
2	1.2	20	13.50		8.40		22.0		1.30		23.30	
3	1.2	40	15.75	15.83	11.45	9.87	27.2	25.70	2.40	1.60	29.60	27.30
4	1.2	40	15.92		8.28		24.2		0.80		25.00	
5	1.2	60	24.53	26.19	8.27	10.96	32.8	37.15	1.37	1.86	34.17	39.01
6	1.2	60	27.85		13.65		41.5		2.35		43.85	
7	1.2	80	26.68	25.64	14.82	12.86	41.5	38.50	2.30	2.65	43.80	41.15
8	1.2	80	24.60		10.90		35.5		3.00		38.50	
9	1.2	100	40.25	35.98	7.95	10.62	48.2	46.60	3.65	4.28	51.85	50.88
10	1.2	100	31.70		13.30		45.0		4.90		49.90	
11	1.2	120	58.40	45.80	4.10	5.45	62.5	51.25	4.00	3.09	66.50	54.34
12	1.2	120	33.20		6.80		40.0		2.18		42.18	
13	1.2	140	43.35	49.52	4.65	4.48	48.0	54.00	1.50	1.90	49.50	55.90
14	1.2	140	55.70		4.30		60.0		2.30		62.30	
15	1.2	160	54.40	55.83	4.30	3.52	58.7	59.35	2.90	3.45	61.60	62.80
16	1.2	160	57.25		2.75		60.0		4.00		64.00	
17	1.2		15.45	16.07	8.55	9.33	24.0	25.40	1.35	1.83	25.35	27.23
18	1.2		16.70		10.10		26.8		2.30		29.10	
19			36.50	27.90	3.00	3.70	39.5	31.60	7.70	8.10	47.20	39.70
20			19.30		4.40		23.7		8.50		32.20	

60 and 80 ton applications are twice as effective as either of the first two and that all the higher applications are about twice as effective as the last two, but nearly equal among themselves. In general, the results show similar tendencies for grain production, except that the difference which we have observed to obtain between the untreated pots and those merely treated with Na_2SO_4, as regards straw production, are not apparent as regards grain production. Just as much grain is produced in pots receiving Na_2SO_4 as in those not receiving any. This is very surprising when we remember how markedly the Na_2SO_4 depressed straw production in the same cultures. The root yields,

however, show much the same effects of the Na_2SO_4 treatment that the grain yields do. Though there are some differences between the two, they are not major differences except possibly for the fact that the protection afforded the straw production by the manure applications is much greater than that afforded the root production. This, of course, might have been expected, owing to the direct contact of the roots with the soil solution.

In the fourth crop, the data for which are set forth in table 8, there is undoubted evidence again of the protective effect of the manure in the directions

TABLE 7

Na_2SO_4 *series. Third crop*

NUMBER	Na_2SO_4 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.2	20	6.50	5.85	2.00	2.40	8.50	8.25	0.50	0.48	9.00	8.73
2	1.2	20	5.20		2.80		8.00		0.46		8.46	
3	1.2	40	5.60	6.05	2.20	2.85	7.80	8.90	1.60	1.40	9.40	10.30
4	1.2	40	6.50		3.50		10.00		1.20		11.20	
5	1.2	60	10.40	10.25	4.40	4.75	14.80	15.00	1.72	1.79	16.52	16.79
6	1.2	60	10.10		5.10		15.20		1.85		17.05	
7	1.2	80	12.85	12.90	7.15	6.30	20.00	19.20	2.20	1.47	22.20	20.67
8	1.2	80	12.95		5.45		18.40		0.74		19.14	
9	1.2	100	30.70	24.35	7.70	9.05	38.40	33.40	1.00	0.80	39.40	34.20
10	1.2	100	18.00		10.40		28.40		0.60		29.00	
11	1.2	120	24.80	25.20	8.60	8.50	33.40	33.70	1.62	1.34	35.02	35.04
12	1.2	120	25.60		8.40		34.00		1.06		35.06	
13	1.2	140	14.50	23.95	5.50	6.85	20.00	30.80	1.50	1.35	21.50	32.15
14	1.2	140	33.40		8.20		41.60		1.20		42.80	
15	1.2	160	28.00	29.70	1.00	3.30	29.00	33.00	1.16	1.68	30.16	34.68
16	1.2	160	31.40		5.60		37.00		2.20		39.20	
17	1.2		3.50	3.60	1.90	1.60	5.40	5.20	0.96	0.81	6.36	6.06
18	1.2		3.70		1.30		5.00		0.66		5.66	
19			12.80	11.95	1.20	1.45	14.00	13.40	3.26	2.48	17.26	15.88
20			15.00		1.00		16.00		2.24		18.24	
21			8.05		2.15		10.20		1.95		12.15	

here considered, and this time as regards straw, grain, and root production. The conditions in the greenhouse were probably better in some way during the growth of the fourth than of the third crop. This seems to have been particularly true for the grain and root production, with special reference to the latter. For some unaccountable reason, root development was far superior in the fourth crop to that in the third and even to that in the second crop. In the fourth crop the 20 and 40 ton applications of manure seem to have lost some of their protective effects, for the yields of straw obtained from

cultures in which they were employed were not even as good as those of the control untreated pots. The duplicate cultures show remarkably good agreement throughout the fourth crop, but it is puzzling to attempt to account for the excellent grain yields which were obtained and especially those of some of the cultures.

It is interesting to note in general throughout the Na_2SO_4 series, what we have already observed in the NaCl series, the tendency for the protective effect of the manure to "wear off" as season follows season. This is well

TABLE 8

Na_2SO_4 *series. Fourth crop*

NUMBER	Na_2SO_4 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm*	*gm.*	*gm.*	*gm.*
1	1.2	20	4.45	4.88	2.65	2.57	7.10	7.45	1.00	1.10	8.10	8.55
2	1.2	20	5.30		2.50		7.80		1.20		9.00	
3	1.2	40	5.90	6.20	3.90	3.70	9.80	9.90	1.40	1.30	11.20	11.20
4	1.2	40	6.50		3.50		10.00		1.20		11.20	
5	1.2	60	9.10	9.25	4.40	4.60	13.50	13.85	2.00	2.00	15.50	15.85
6	1.2	60	9.40		4.80		14.20		2.00		16.20	
7	1.2	80	10.00	9.95	6.30	6.20	16.30	16.15	2.40	2.25	18.70	18.40
8	1.2	80	9.90		6.10		16.00		2.10		18.10	
9	1.2	100	13.50	14.75	7.30	7.90	20.80	22.65	Lost	5.00	20.80	24.90
10	1.2	100	16.00		8.50		24.50		5.00		29.00	
11	1.2	120	13.30	15.25	8.70	10.35	22.00	25.60	3.40	3.20	25.40	28.80
12	1.2	120	17.20		12.00		29.20		3.00		32.20	
13	1.2	140	42.60	43.10	25.40	23.50	68.00	66.60	8.40	9.20	76.40	75.80
14	1.2	140	43.60		21.60		65.20		10.00		75.20	
15	1.2	160	46.40	46.50	20.90	20.65	67.30	67.15	8.00	9.00	75.30	76.15
16	1.2	160	46.60		20.40		67.00		10.00		77.00	
17	1.2		4.25	4.40	2.45	2.35	6.70	6.75	0.90	0.95	7.60	7.70
18	1.2		4.55		2.25		6.80		1.00		7.80	
19			7.30	7.80	2.70	3.73	10.05	11.53	4.50	5.17	14.55	16.70
20			8.90		4.20		13.10		7.00		20.10	
21			7.20		4.30		11.50		4.00		15.50	

exemplified by the longer period of protection afforded by the larger than by the smaller manure applications, and the protective effect seems to increase in direct proportion to the increase in the quantity of manure employed. This statement needs some qualification of course, but a study of the data will make clear to the reader the evident tendency which is called to his attention. The reason for the superior protective effect of the manure to the roots in the fourth to that in the third crop does not seem to us evident at the present time.

Series 3. Na_2CO_3

The experiment with the Na_2CO_3 was conducted in the same four seasons as those with the other salts and parallel with them. Only three crops were obtained in series 3, however, since following the second application of Na_2CO_3 (prior to the second crop) the physical condition of the soil was so badly affected by the salt and its effects and the hydroxyl ion concentration probably was so great that the seed planted for the second crop would not germinate. The

TABLE 9

Na_2CO_3 *series. First crop*

NUMBER	Na_2CO_3 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.3	20	22.8	22.90			22.8	22.90	1.80	1.80	24.60	23.80
2	0.3	20	23.0				23.0				23.00	
3	0.3	40	30.0	34.40			30.0	34.40	3.50	3.10	33.50	37.50
4	0.3	40	38.8				38.8		2.70		41.50	
5	0.3	60	49.5	41.75			49.5	41.75	5.50	4.75	55.00	46.50
6	0.3	60	34.0				34.0		4.00		38.00	
7	0.3	80	38.2	39.85			38.2	39.85	6.50	5.70	44.70	45.55
8	0.3	80	41.5				41.5		4.90		46.40	
9	0.3	100	42.5	41.70			42.5	41.70	6.20	4.70	48.70	46.40
10	0.3	100	40.9				40.9		3.20		44.10	
11	0.3	120	54.0	45.00			54.0	45.00	5.20	4.60	59.20	49.60
12	0.3	120	36.0				36.0		4.00		40.00	
13	0.3	140	47.8	49.30			47.8	49.30	(11.50)	5.75	53.55	55.05
14	0.3	140	50.8				50.8				56.55	
15	0.3	160	46.6	44.60			46.6	44.60	6.20	6.10	52.80	50.70
16	0.3	160	42.6				42.6		6.00		48.60	
17	0.3		10.0	9.00			10.0	9.00	1.00	1.20	11.00	10.20
18	0.3		8.0				8.0		1.40		9.40	
19			47.0	42.50			47.0	42.50	6.70	7.97	53.70	50.47
20			40.0				40.0		11.70		51.70	
21			40.5				40.5		5.50		46.00	

results of series 3 are set forth, therefore, in three tables, viz., tables 9, 10, and 11, which give the yields of straw, grain, and roots respectively in the first, third, and fourth crops wherever they were obtained.

The data of the first crop in this series are probably the only significant ones, since in spite of the doubling of the amount of salt prior to seeding the second crop, the Na_2CO_3 seemed to have lost its toxicity in the third and fourth crop, at least in so far as straw and grain production are concerned. The root yields are unquestionably injured by the effects of the Na_2CO_3

application even in the third and fourth crops, as shown by comparison of the data for the control untreated pots with those for the Na_2CO_3 treated pots and of both of these with the manure treated pots. In view of the foregoing statements, we may dispose of tables 10 and 11 without further comment. A word more may be necessary, however, with respect to table 9. In the latter, which represents the first crop in which only straw and root yields were obtained, the evidence seems to point unquestionably to the injurious effect of 0.3 per cent Na_2CO_3 on both straw and root production of

TABLE 10

Na_2CO_3 *series. Third crop*

NUMBER	Na_2CO_3 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	19.45	18.58	3.15	3.22	22.60	21.80	0.30	0.33	22.90	22.13
2	0.6	20	17.70		3.30		21.00		0.35		21.35	
3	0.6	40		17.95		6.85		24.80		0.60		25.40
4	0.6	40	17.95		6.85		24.80				25.40	
5	0.6	60	31.20	32.80	1.00	2.80	32.20	35.60	0.67	0.74	32.87	36.34
6	0.6	60	34.40		4.60		39.00		0.80		39.80	
7	0.6	80	47.00	42.25	0.40	1.95	47.40	44.20	1.60	1.30	49.00	45.50
8	0.6	80	37.50		3.50		41.00		1.00		42.00	
9	0.6	100	29.95	41.07	3.65	3.53	33.60	44.60	2.00	1.59	35.60	46.19
10	0.6	100	52.20		3.40		55.60		1.18		56.78	
11	0.6	120	32.50	34.80	2.50	2.00	35.00	36.80	1.02	1.02	36.02	37.82
12	0.6	120	37.10		1.50		38.60		1.02		39.62	
13	0.6	140	33.54	43.37	2.86	1.83	36.40	45.20	1.46	1.77	37.86	46.97
14	0.6	140	53.20		0.80		54.00		2.08		56.08	
15	0.6	160	32.00	32.60	4.00	3.90	36.00	36.50	1.70	1.75	37.70	38.25
16	0.6	160	33.20		3.80		37.00		1.80		38.80	
17	0.6		15.40	14.00	4.60	3.00	20.00	17.00	0.57	0.57	20.00	17.57
18	0.6		12.60		1.40		14.00		Lost		14.00	
19			15.00	11.95	1.00	1.45	16.00	13.40	2.24	2.48	18.24	15.88
20			12.80		1.20		14.00		3.26		17.26	
21			8.05		2.15		10.20		1.95		12.15	

barley on that soil. The yield of straw on the Na_2CO_3 treated pots was only about one-fifth as great as on the untreated control pots, and the yield of roots only about one-seventh as great in the same pots. The evidence is also clear in that table that manure exerts a strikingly protective effect on the barley plant as regards both straw and root production. Even the 20 ton manure application is markedly effective so far as straw production is concerned, and with the 60 ton application the yields are practically as good as in the pots receiving no alkali. The other features of table 9 and of the Na_2CO_3 series, in general, speak for themselves.

TABLE 11

Na_2CO_3 *series. Fourth crop*

NUMBER	Na_2CO_3 ADDED TO SOIL	MANURE RATE PER ACRE	WEIGHT OF STRAW	AVERAGE WEIGHT OF STRAW	WEIGHT OF GRAIN	AVERAGE WEIGHT OF GRAIN	WEIGHT TOTAL DRY MATTER ABOVE SURFACE	AVERAGE WEIGHT TOTAL DRY MATTER ABOVE SURFACE	WEIGHT OF ROOTS	AVERAGE WEIGHT OF ROOTS	WEIGHT TOTAL DRY MATTER	AVERAGE WEIGHT OF TOTAL DRY MATTER
	per cent	*tons*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	0.6	20	7.20	7.90	3.70	3.80	10.90	11.70	1.00	1.20	11.90	12.90
2	0.6	20	8.60		3.90		10.50		1.40		13.90	
3	0.6	40		8.30		3.50		11.80		0.60		12.40
4	0.6	40	8.30		3.50		11.80		0.60		12.40	
5	0.6	60	24.50	24.55	8.00	7.70	32.50	32.25	1.40	1.45	33.90	33.70
6	0.6	60	24.60		7.40		32.00		1.50		33.50	
7	0.6	80	47.40	29.40	14.10	10.35	61.50	39.75	1.00	0.75	62.50	40.50
8*	0.6	80	11.40		6.60		18.00		0.50		18.50	
9	0.6	100	38.00	30.90	12.00	11.85	50.00	32.75	1.20	2.50	51.20	45.25
10	0.6	100	23.80		11.70		35.50		3.80		39.30	
11	0.6	120	15.60	24.75	10.20	7.50	25.80	32.25	1.50	1.40	27.30	33.65
12	0.6	120	33.90		4.80		38.70		1.30		40.00	
13	0.6	140	31.20	34.65	15.60	12.25	46.80	46.90	4.00	3.75	50.80	50.65
14	0.6	140	38.10		8.90		47.00		3.50		50.50	
15	0.6	160	50.80	55.50	19.50	15.08	70.30	70.55	3.70	4.85	74.00	75.40
16	0.6	160	60.20		10.60		70.80		6.00		76.80	
17	0.6		7.40	6.20	3.40	3.45	10.80	9.65	0.30	0.40	11.10	10.05
18	0.6		5.00		3.50		8.50		0.50		9.00	
19			7.30	7.80	2.70	3.73	10.00	11.53	4.50	5.17	14.50	16.70
20			8.90		4.20		13.10		7.00		20.10	
21			7.20		4.30		11.50		4.00		15.50	

*Spoiled by rain.

GENERAL DISCUSSION

The data presented in the tables and the comments made on them in the discussion having been studied it becomes pertinent to inquire into some of the general bearings of the investigation and its significance, as well as into the nature of the causes which may have contributed to the results obtained. It is interesting first to note how great a tolerance or partial tolerance the barley plant exhibits in the clay adobe soil for the common alkali salts which are used singly. It is important to specify that this is true for this clay adobe soil, since, as many earlier publications have shown, soils vary considerably with regard to their powers to render innocuous more or less of the alkali salts to plants. We speak of this here because this fact has a significant bearing on the theoretical side of our investigation. Soils with a large internal surface always have shown themselves superior to those with a relatively small internal surface in their powers to inhibit the injurious effects of salts on plant growth. The considerable, even striking, resistance of the barley plant to the effects of single alkali salts which we have noted above are

therefore, in all probability, to be ascribed in very large measure to the physical nature of the soil used.

But, as we have shown, even such considerable tolerance of the barley plant to toxic effects of salts is very much enhanced by the addition of organic matter in the form of barnyard manure. It is well known that the rapid decay which manure undergoes in most soils is accompanied by the production of very large quantities of organic colloids. The latter must, of course, increase in very large degree the total internal soil surface. Hence, it is natural to conclude that much, if not all, of the protective effect afforded plants by the incorporation of manure into the soil in which they are grown and which contains alkali is due to the increase of surface in the soil following upon not only the decay of the manure but to some extent also on the mere introduction of the manure into the soil.

The probable mechanism of the action of internal soil surface in inhibiting the toxic effects of salts on plants has been discussed in other papers issued from this laboratory, but may be briefly discussed here again. From long study and on theoretical grounds, we have arrived at the conclusion that the internal surface of soils exerts the effects in question largely by removing from active participation in the actually available soil solution a certain amount of the salt through adsorption—a physical phenomenon well recognized but not clearly understood. The increase in soil surface, therefore, resulting from the addition of organic colloids (in this case from the decay of manure) virtually removes from the sphere of usable soil solution a more or less considerable part of the salt which the normal soil surface, prior to the introduction of the additional colloids, had not removed therefrom. We believe this to be the most important phase of the protective effect of manure in soils containing much soluble salt. Nevertheless, we do not deny that other agencies brought into play by the decomposition of the manure may exert important influences in the same general direction. The chemical reactions occurring between the soluble constituents of the manure and the salt, the changes following therefrom which result in the different balancing of the nutrient and non-nutrient elements of the soil solution, the stimulating effect of constituents of the manure on the plant directly, and indirectly through similar effects on the soil bacteria and solvent effects on the soil minerals, are all doubtless, among other factors, of considerable significance to our problem in the very complicated medium resulting from the mixture of the soil and manure.

Turning now from the theoretical to the practical phases of the question studied in this paper, we should like to direct attention to the following. With all the defects and inaccuracies of such an investigation as that which we have carried out taken into consideration, and with the inherent difficulties which characterize soil and plant problems recognized, there seems still to remain, from a study of our data, the conclusion that manure may be used, if available in sufficient quantity, to render more or less harmless, to plants,

injurious alkali salts in soils. The degree to which manure will thus act depends on the amount of internal surface already possessed by the soil and the amount added by the manure. It is further contingent upon the nature and amount of the manure and the chemical nature of the salts of the soil. It appears now that nothing short of heavy applications of manure to soil will be effective. This may mean applications varying from 10 to 40 tons per acre, depending on the kind of soil and salt and the amounts of the latter present. The protective action of the manure is not permanent but ephemeral, the length of time for which it is effective varying with the amount used and probably with the other factors considered above. It remains true, nevertheless, that on much of the alkali land of the arid west, which contains less salts than the amounts used in our experiments, it should be feasible and profitable to offset the inhibiting effects of the salts on crop growth partly or wholly by the use of barnyard manure, or stable manure and possibly by other forms of organic matter. In connection with this statement, it should be observed that on similar grounds green manure crops, straw, hay, or any form of non-toxic organic matter which will decay with some degree of facility will serve as substitutes for manure and will be as effective as other conditions, which are above discussed, will permit. Our experiments do not furnish any evidence on the effect of manure on mixtures of salts in soils, but previous experiments by the senior author and L. T. Sharp in pot work (1) and in the field (2) have in general given similar results to those above discussed. Needless to say, many more theoretical and practical investigations are needed which will shed further light on this fascinating problem, and they will be forthcoming.

SUMMARY AND CONCLUSION

The authors have conducted an experiment in the greenhouse in pot cultures to determine how manure applications affect soil containing soluble salts as a medium for barley growth. Four successive crops were grown. The salts were tested out singly at rates of 0.3 per cent each NaCl and Na_2CO_3 and 0.6 per cent Na_2SO_4 based on the dry weight of the soil. Additional quantities of salts were added at the same rate prior to planting the second crop and the last three crops were thus grown in the presence of the larger quantities of salts. No conclusions without some qualification can be drawn. The reader is therefore urged to peruse the discussion above. Nevertheless, our data point to the undoubted protective effect exerted by manure in soil for barley plants grown in the presence of alkali salts. Culture pots containing NaCl and Na_2SO_4 give more definite results than those containing Na_2CO_3. It seems fairly certain that our results point to a practical application of considerable value.

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THE ACTION OF SOME COMMON SOIL AMENDMENTS

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That some fertilizers have an indirect effect has long been an acknowledged fact. It is no longer believed that the increase in yield obtained from the use of one ton of barnyard manure is alone due to the addition of the two or three pounds of phosphorus and the ten or twelve pounds each of nitrogen and potassium which it contains; but no small part of the increase is due to the liberation of more plant food. This (139) is brought about by the decaying of organic matter with the formation of various acids which in turn act as solvents on insoluble plant-food already in the soil. The benefits obtained from the use of gypsum and lime are due not only to the neutralizing of acid and supplying of calcium but it was believed that they act indirectly by liberating potassium. From certain results obtained in various experiments, which will be considered later it seems as if this may be due in part to the rapid mineralization of the soil nitrogen occurring in the presence of these compounds.

The theory of the liberation of plant-food by the addition of various soluble salts to the soil is not a new one, for some writers speak of it as an established fact. This was understood in a general way by Justus von Liebig, as early as 1856, for in his article (65), "Some Points in Agricultural Chemistry," he says: "These salts (speaking of the ammonium salts) contain an acid which exerts an action on the constituents of the soil, an action which is not exerted by pure ammonia. The acids of the ammoniacal salts render the earthy phosphates more soluble in water than they would otherwise be."

A. Stood (137), in a study of the effects of salt water on the soil, attributes part of the bad effects of salt water on land to the rendering soluble of the phosphates which are subsequently washed beyond the plant roots.

For some time in England the agricultural investigators were divided into two schools. One of these claimed that a great increase in yield could be obtained by the use of an insoluble phosphate, while the other claimed that very little—if any—increase in yield could be obtained by its use. Each supported his claim by actual field tests. This difference in results was explained by E. Wildt (162) as being due to the salt which in some cases had been used in connection with the phosphate. Some salts, he claims, when used in connection with a phosphate tend to render it more soluble. Especially does he attribute this property to the various nitrates.

Coming down to the present time we have the statements of Hilgard (46) that lower percentages of potassium, phosphorus, and nitrogen are adequate

when a large proportion of lime carbonate is present. He states further that a high percentage of lime carbonate may offset a small percentage of phosphorus, apparently by bringing about greater availability. Again, in summing up the chemical actions of carbonates he states that carbonates liberate phosphorus and potassium from insoluble forms.

Wagner (156) found that some of the benefits which result from the use of sodium nitrate are due to its rendering more soluble certain phosphates.

SALTS CONTAINING NITROGEN COMPARED WITH DRIED BLOOD AS A SOURCE OF NITROGEN

As a source of nitrogen the nitrate of soda is usually considered to produce a much better yield than an equivalent amount in the form of dried blood. This is well illustrated in a series of pot experiments by Voorhees (154). The experiments were carried out in the years 1901 and 1902. Sodium nitrate and dried blood were the sources of nitrogen, equivalent amounts of nitrogen being used in each case. As an average of two years and of three experiments each year, dried blood gave a yield of 106.8 gm., while sodium nitrate yielded 110.4 gm. of dry plant.

Patterson (94) obtained similar results with field tests as the following table will show. Each result is the average of two years' work.

TABLE 1

Yield of grain and hay with various nitrogenous fertilizers

CROP	FERTILIZER	GRAIN	FODDER
		bushels	*pounds*
Corn	Sodium nitrate	61.5	3050
Corn	Ammonium sulfate	52.7	2625
Corn	Dried blood	49.7	2812
Wheat	Sodium nitrate	14.9	2030
Wheat	Ammonium sulfate	14.3	1830
Wheat	Dried blood	9.0	884
Hay	Sodium nitrate		4150
Hay	Ammonium sulfate		1900
Hay	Dried blood		2550

The above table shows that corn with sodium nitrate yielded 11.8 bushels and with ammonium sulfate 3 bushels more than it did with an equivalent amount of nitrogen in the form of dried blood. Wheat with sodium nitrate yielded 5.9 bushels and with ammonium sulfate 5.3 bushels more than with the dried blood. Hay produced 1600 pounds more with sodium nitrate and 654 pounds less with the ammonium sulfate than with the dried blood.

A. Müntz (87) also showed that sodium nitrate produced better yields than an equivalent amount of nitrogen in the form of dried blood.

Therefore, under ordinary conditions sodium nitrate is more effective than ammonium sulfate, which, however, is more effective than an equivalent amount of dried blood. This, however, in the case of sodium nitrate and ammonium sulfate is reversed under certain conditions, as will be shown in the following discussion.

The relative value of sodium nitrate and ammonium sulfate when used in connection with an insoluble phosphate

It seems likely that this difference in value of the two fertilizers may be due in part to some indirect effect of the salt. This is at least indicated by the difference in action of ammonium salts and sodium nitrate when used in connection with insoluble phosphate. On ordinary soil sodium nitrate is usually conceded to give the better yield (140). However, when an insoluble phosphate is used in connection with the nitrogenous manure the ammonium salt gives a larger yield than the sodium nitrate or nitrogen from organic sources. Besides, plants grown with the ammonium salts contain a larger percentage of phosphorus.

A great number of experiments have been carried out to ascertain the relative value of insoluble phosphates. A few of them are as follows: Jameson (48) conducted a series of experiments with turnips in which a soluble and insoluble phosphate was used. In one series a soluble salt was used with the phosphates, in the other phosphates were used alone. As an average of forty experiments he obtained with the soluble phosphate 15,133 kilos per acre, while with the insoluble phosphate he obtained 14,663 kilos per acre. This is a difference of 470 kilos in favor of the soluble phosphate. When the same phosphates were used in connection with ammonium sulfate in a series of 12 experiments, the yield was as great with the insoluble as with the soluble phosphate. Now, when the same two phosphates were used in connection with sodium nitrate the soluble gave a yield of 22,240 kilos, while the insoluble gave but 20,525 kilos. It may be seen that there was 1715 kilos per acre in favor of the soluble phosphate. When the above facts are taken into consideration, remembering that nitrates are usually the best form of nitrogen, it would seem that the ammonium sulfate had some effect on the insoluble phosphate.

Krocker and Grahl (59) obtained as large a yield with insoluble phosphate as with soluble phosphates when ammonium sulfate was used in connection with the phosphate.

A number of experimenters have noted the above facts and carried out various experiments to ascertain the nature of this effect. One of the prominent workers in this field is Soderbaum (130), from whose work the following table was taken. The crop grown was oats.

The yield with the bone meal and ammonium sulfate was as great as with the soluble phosphate and sodium nitrate. While not so good as ammonium sulfate, ammonium nitrate gives a better yield than sodium nitrate or the or-

ganic manures. It may be seen that when used with slightly soluble phosphate sodium nitrate is no better than the organic manure.

Thes results have been fully confirmed by more recent experiments by the same worker (130–132) and others, (17, 119).

This greater yield with the ammonium salts has been attributed to a "physiological action" of the ammonium sulfate on the plant, and not to a solvent action on the insoluble plant food. Nedokuchaev (88) found that ammonium sulfate invariably increased the yield over that obtained with other sources of nitrogen, and he considered it due to the sulfate rendering the phosphorus, more soluble and the experiment of Schulov (115) indicates this to be the cause. In these experiments there were two sets of pots, one set in which the ammonium salts and the phosphate were thoroughly mixed, while in the other set the ammonium salts and phosphate were separate but both were accessible to the plant. Where the fertilizers were mixed there was much larger yield obtained with the ammonium nitrate than with the sodium nitrate. However,

TABLE 2

Yield of grain with various fertilizers

FERTILIZER	YIELD OF GRAIN
	gm.
Soluble phosphate	16.1
Soluble phosphate + sodium nitrate	61.9
Bone meal + sodium nitrate	49.4
Bone meal + ammonium nitrate	57.9
Bone meal + ammonium sulfate + sodium nitrate	55.9
Bone meal + ammonium sulfate	62.9
Bone meal + urea	53.1
Bone meal + urea + albumin	51.1

where the nitrogen and phosphorus were separate the yield with each fertilizer was the same.

The work of Brooks (7), at the Hatch Experiment Station on the comparative value of potassium chlorid and potassium sulfate, is of interest in this connection. In a 3-year test on potatoes, there was an average of 22.1 bushels more where the sulfate was used than where the chlorid was used.

We must bear in mind that this beneficial effect of the sulfate may be due in part to the plant-food which it supplies in the form of sulfur. That plants require the presence of sulfur to make a healthy growth is well known. Bogranov, Haseloff and Goosel, König and others have found that part of the beneficial effects obtained from the use of a sulfate is due to the sulfur which acts as a plant food. With Brook's experiment, however, there was an increase in the starch in the potato, and Seisl and Gross (120) found that leaves of potatoes which were rich in starch invariably contained more potassium and phosphorus than ones low in this constituent.

That the increase is not due entirely to the action of the sulfur as a plant-food is further shown when we make a study of the phosphorus in the plants which have been grown with various fertilizers. A Swedish investigator, Weihall, (158) found that oats grown with an ammonium salt contained 0.397 per cent of phosphorus, whereas those grown without the ammonium salt contained 0.375 per cent phosphorus. Prianishnikov (105) found that buckwheat grown with insoluble phosphate and sodium nitrate contained 0.105 per cent of phosphorus,while that grown with the same phosphate, in connection with ammonium nitrate, contained 0.253 per cent phosphorus. Barley grown with ammonium nitrate contained 0.10 per cent more phosphorus than that grown with sodium nitrate.

The experiment which illustrates this best is the mixed herbage of permanent grass land by Lawes and Gilbert (62). As an average of eighteen years, the plat which receives no manure had 2.288 per cent phosphorus in the ash of the grass, while that which received ammonium salts had 2.790 per cent. This is 0.502 per cent more phosphorus in the ash of the plants which had received ammonium salts. In addition to this increase there was a greater yield of hay on the manured than on the unmanured, so that the total phosphorus taken up by the plant would be considerably more in the one case than in the other.

Again, take the plat in the Rothamsted experiment (113) with wheat which received ammonium salts, and compare it with the unmanured plat. The plat which received no manure produced as an average of 42 years $12\frac{3}{4}$ bushels of wheat per acre, while the one receiving ammonium salts averaged $19\frac{1}{2}$ bushels per acre. We find that 11.64 pounds of phosphorus were taken up by the crop on the unmanured land, while the one receiving ammonium salts gave as an average 14.5 pounds per acre. From this we see that there were 2.93 pounds per acre more taken up where the ammonium salts had been used. However, in this case the percentage composition is no higher in the manured grain.

That the phosphorus is more soluble on the plats which have been manured with ammonium salts is further shown by a comparison of the drainage water of the two plats. The unmanured plat had as an average 0.275 parts per million of phosphorus in the drainage water. The plat which received ammonium salts had as an average 0.629 parts per million of phosphorus, thus showing that the drainage water from the manured plat is richer in phosphorus than from the unmanured.

The above facts show that of the nitrogen compounds, ammonium nitrate is the most effective in causing the assimilation of phosphorus from insoluble phosphates. The ammonium sulfate stands next, while sodium nitrate has little if any effect. This is well illustrated by the work of Prianishnikov (104) who made some very thorough tests in which he used phosphorite with various nitrogenous salts. The following table gives the results he obtained with oats:

TABLE 3

Yield of oats, and phosphorus in the oats, with various fertilizers

SOURCE OF NITROGEN	PHOSPHORITE						KH_2PO_4
	$NaNO_3$	$\frac{3}{4}$ $NaNO_3$ $\frac{1}{4}$ $(NH_4)_2SO_4$	$\frac{1}{2}$ $NaNO_3$ $\frac{1}{2}$ $(NH_3)_2SO_4$	$\frac{1}{4}$ $NaNO_3$ $\frac{3}{4}$ $(NH_4)_2SO_4$	$(NH_4)_2SO_4$	NH_4NO_3	$NaNO_3$
Yield (gm.).....	6.900	22.000	20.500	19.200	1.600	18.90	19.800
Per cent phosphorus.	0.039	0.131	0.249	0.402	0.637	0.244	0.231
Total phosphorus in plant (mgm.).....	2.707	30.815	50.990	77.000	9.210	46.02	45.750

The yield was as great with the ammonium nitrate and phosphorite as it was with the soluble phosphate and sodium nitrate. The percentage of phosphorus in the two crops was also the same. Similar tests were made using barley, buckwheat, peas, flax, vetch, and in every case where the ammonium nitrate was used with the phosphate the yield was practically as large as it was with the soluble phosphate. The percentage of phosphorus in the plant was also high with ammonium salts. Thinking this may be due to nitrification, he carried on tests in sterile cultures where nitrification did not occur and found that even then the ammonium nitrate increased the assimilability of phosphorus of insoluble phosphates.

He (106–109) did, however, find that when ammonium sulfate was used alone on some soils the plants were injured by the "physiological action" of the acid condition produced in the soil, although they were super-saturated with phosphorus. The injury from this cause was reduced by the application of small quantities (0.25 per cent) of calcium carbonate, and the yield was increased. When the amount of calcium carbonate was increased to more than 1 per cent all of the free sulfuric acid was neutralized and the plant suffered from phosphorus starvation. The ammonium sulfate not only increased the yield but the phosphorus content of the plants. The difference in the quantity of phosphorus of the soil would account for the various results reported by different workers (43, 147), although it might be assumed that the marked physiological alkalinity of calcium nitrate would depress the assimilation of phosphorus. Yet there are experiments (117) in which the solubility of phosphorus has been increased by it. This must be due to the action of the calcium nitrate on the biologic processes going on in the soil.

Sodium chloride as a fertilizer

Sodium chloride, when used as a fertilizer, varies under different conditions. Some experimenters obtained a good yield from its use, others obtain just as good a yield without it. There must be some cause for this difference and it may be due to its indirect effects on other plant-food either direct or indirect through its action on bacteria.

Storp (141), in an article on sodium chloride as a manure, attributes the benefit derived from its use as due to its decomposing insoluble plant-food. If this be the correct theory we can account for yields such as those obtained by Voelcker (153). As an average of five experiments, on land which had been manured with common salt, the yield of mangels was 36,060 pounds. On the adjoining unmanured ground there was but 26,035 pounds, a difference of a little over 10,000 pounds due to the use of common salt. Now, if the land were rich in insoluble plant-food and the choloride were able to liberate it, we could expect a larger yield. On the other hand, if the soil had been poor in unavailable plant-food no good result would have followed its use. Wheeler (157) seems to have established the fact that sodium chloride cannot to any great extent take the place of potassium salts. However, he does think that sodium chloride can liberate phosphorus from insoluble forms as the following will indicate: "It may, however, be stated here that sodium salts seem to liberate phosphorus and potassium so that under certain circumstances they may act as an indirect manure." In another report (161) he shows that the percentage of phosphorus in a plant is increased by the use of a sodium salt. With radishes this was in some cases as much as 0.052 per cent more in the crop from land which had received a full ration of sodium over that which received but a part ration. In the case of turnips there was a difference of 0.121 per cent, of beets 0.035 per cent, of carrots 0.074 per cent, whereas in the case of the chickory the results are practically the same in the crop from the manured and unmanured land. The report contains many more cases in which sodium chloride increased the phosphorus in the plant.

Most clays, clay soils, muck, and some other soils yield acid solutions when extracted with sodium chlorid, and hence would be able to dissolve insoluble phosphates (86). It is significant that the sodium salts when applied to a soil often not only increase the yield but markedly increase the nitogen content of the crop (151). Ewart (25) considers that the stimulating action of a moderate dressing of sodium chloride is due partly to its solvent action on the mineral constituents of the soil. But Schulze (118) considers that common salt solutions do not have a solvent effect on potassium zeolites, and that if an increase in crop is obtained by fertilizing with common salt it cannot be attributed to the indirect effect of the salt in setting free the potash of potassium zeolites in the soil.

It is quite evident from the preceding consideration that common salt often increase the yield of beets, mangels, barley, wheat, and some other crops. Moreover, it is certain that this increase is due in a large measure to the increased available nitrogen and phosphorus of the soil.

Calcium carbonate on the solubility of plant-food

It is usually conceded that a calcareous soil is exceptionally productive and the soil constituents become available more readily than in the absence of cal-

cium carbonate. Yet there are experiments reported in which calcium carbonate exerted a depressing action on the assimilability of phosphorus from aluminum and iron phosphate (108), raw phosphate, bone meal, and tricalcium phosphate (107–109). Some workers (121) have even found that the addition of calcium carbonate to iron phosphate reduced its solubility in acetic acid and its effectiveness as a food for plants grown in pots. There are, however, experimenters who have found calcium carbonate to increase the availability of aluminum and iron phosphates (33) and Simmermacher (126) found that calcium carbonate does not reduce the fertilizing value of even monocalcium phosphate.

Physically, however, even a small amount of lime carbonate by its solubility in the carbonated soil water will act beneficially in causing the flocculation of clay and in the subsequent conservation of the flocculent or tilth condition, by acting as a light cement holding the soil crumbs together when the capillary water has evaporated, thus favoring the penetration of both water and air. This would enhance bacterial activity such as nitrification (8), nitrogen fixation, and sulfofication (11). It also increases in general the rate of decomposition of organic substances in the soil (63). The increased bacterial activity thus occurring would result in the production of large quantities of organic acids which would react with the insoluble plant-food of the soil rendering it soluble. This would account for the more available phosphorus of the soil noted by some workers (30).

Effects of lime on phosphorus

It seems to be a well-established fact that lime will, under certain conditions, liberate phosphorus from the soil. The more recent work on this subject is that of the Rhode Island Experiment Station. Hartwell and Kellogg (42) in speaking of their turnip experiments, with and without lime say:

> The crop of turnip roots from the limed plat which had received finely ground bone was 62 per cent greater than from the corresponding unlimed plat and the per cent of phosphorus in the dry matter of the roots was 0.378 from the limed plat and 0.351 from the unlimed one. Again, the increase in the crop of turnip roots from the limed plat to which slag meal had been added was 34 per cent as compared with the unlimed plat and phosphorus in the dry matter of the roots was 0.324 per cent from the lime plat and 0.309 per cent from the unlimed one. These increases in the percentage of phosphorus in the turnip roots grown upon the limed plats furnish some evidence that more of the phosphorus in the plats was assimilable.

These same authors made a test of the phosphorus in the soil by extracting with and without lime. They found more phosphorus in the solution from the soil which had been treated with lime than that which had not.

In this same line are the experiments of Kellner (55) and his co-workers. They found in the field and laboratory test that phosphorus was liberated by the use of lime.

Again, the work of Sutherst (142) shows that insoluble phosphates of the soil become much more soluble when treated with lime. Especially was this true in the case of the ferric phosphate. The solvent action was not found to take place when calcium carbonate was used.

The work of Wheeler and Adams, in "A Test of Nine Phosphates with Different Plants," is full of illustrations in which lime has been effective in the liberation of phosphorus. They even claim that it may be of value when a soluble phosphate is used, as may be seen from the following:

> The results seem to indicate that in a soil deficient or devoid of carbonate of lime and well supplied with the oxides of iron and aluminum, liming may extend the period of efficiency of the soluble phosphates possible by combining with much of the phosphorus at once, and thus holding it in more assimilable combinations than if it were possible for it all to unite immediately with the iron and aluminum oxides.

Lime may increase the solubility of phosphorus in the soil by replacing iron and aluminum, which is in combination with the phosphorus. It does not, however, increase the soluble potassium of the soil according to Garther (32). Vincent (149) points out that while granite soils are generally supposed to be poor in phosphorus since they respond to applications of phosphorus, the application of lime or chalk has a similar effect. This he attributes to the existence of the phosphorus in unavailable organic combinations with humus which must be neutralized before the phosphorus becomes available.

Calcium sulfate

Calcium sulfate is the most powerful soil stimulant we have. This is due mainly to its liberation of plant-food, especially potassium (72). However, there are soils wherein it does not increase the solubility of the potassium (6) and still there is a response in better crops when gypsum is applied to the soil.

The experiments carried on at Tokyo (50) show that rice yielded better and had a better color when grown on land manured with gypsum.

The analyses made by Boussingault and quoted by Storers (138) shows a greater amount of phosphorus in clover taken from land manured with gypsum. The phosphorus in the clover from the manured land was 10.57 kilos; that from the unmanured 4.80 kilos. The following year, although no more manure was applied, the phosphorus from the hay grown on the manured land was 6.93 kilos more than from the unmanured.

Pfeffer (98) states that Knop found that when seeds are in water containing calcium sulfate, the calcium of the salt is absorbed in a somewhat greater amount than the acid. If this be true it is easy to see how calcium sulfate can assist in the assimilation of phosphorus, even though the phosphates are found to be less soluble in a calcium sulfate solution.

Moreover, the addition of gypsum to a soil often increases the total nitrogen of the crop removed from such a soil (75), and it is well known that gypsum increases very materially the ammonifying, nitrifying, and sulfofying powers of the soil (11).

Iron sulfate

Some writers have made great claims for iron sulfate as a fertilizer. A goodly number of these claims have been made by persons who would profit by its sale. Even when we ignore these cases, there are still cases in which it has produced good results.

The man who made the greatest claim for this, and backed his claim with actual field tests, was Griffiths (40). He made tests with it as a manure on a number of crops. The yields which he obtained were much greater with than without. Especially was this true with beans, turnips mangels, potatoes, meadow hay, and grass. With wheat and other grains the yield did not appear to increase with the application of the iron sulfate. Griffiths attributed this increase in yield to the iron supplied to the plant. For he found considerably more iron in the plants which had been grown on soil manured with iron sulfate than those grown on the unmanured soil. The increase may be due in part to this cause, but a study of the phosphorus of the crop indicates that there is also another cause.

As an average of three years the bean plants grown on land manured with iron sulfate contained 17.95 per cent (40) of phosphorus in the ash, while those grown on adjoining unmanured land contained but 16.47 per cent of phosphorus. In the ash of the pods alone, there was 15.78 per cent phosphoric acid in those from the manured land and 15.03 from the unmanured. The phosphorus in the seed from the manured and unmanured land was the same. With turnip leaves it stood 3.03 per cent in the ash of those grown with the manure and 1.84 per cent in those grown without it. In the roots there was 0.61 per cent more phosphorus in the ash of those grown with sulfate than in those grown without it. Meadow hay had 3.39 per cent phosphorus in the ash of that grown on land manured with the sulfate and 2.34 per cent in that grown without it. Practically the same relationship exists in grass grown under the two conditions. Mangels, 1.00 per cent, potatoes, 1.01 per cent, beet roots, 1.18 per cent more in the ash of those grown on land manured with sulfate than those grown on land not thus manured. Wheat was about the same on manured and unmanured land.

Boetet and Paturel obtained an increase in the crop due to the use of iron sulfate, but they differ from Griffiths in not finding a greater amount of iron in the plants grown on land manured with iron sulfate.

The Hill (150) experiments in England are of the same type. Wheat was grown in pot experiments with and without iron sulfate. The pot which received no sulfate yielded 35.28 gm. of wheat and straw, while an average of the three manured pots was 36.48 gm. The yield was greatest on the pot which received at the rate of 100 pounds of iron sulfate per acre.

Brooks (7) obtained a larger yield of soybeans on land manured with iron sulfate than on unmanured land. However, he did not find a deeper green in the plants on the manured land as did Griffiths.

Lipman (75) found as an average of the results of four years' plat experiments with corn, oats, wheat and timothy, using ferrous sulfate at the rates of 50, 100, and 200 pounds per acre that there was in every an increase in dry matter and total nitrogen of the crop. That the increased nitrogen content of the plant resulted from increased bacterial activity would seem likely from the results reported by Vermarel and Danthony (148) who found that iron pyrites increased the yields of wheat and beans 30 to 60 per cent when used in combination with organic matter. They were without effect when used on soils lacking in organic matter and receiving nitrogen in the form of sodium nitrate.

Other sulfates

The sulfates seem to act very strongly on the insoluble phosphorus of the soil. Where there is a lack of available phosphorus, the sulfates produce yields over and above the chlorids or nitrates with the exception of ammonium nitrate. The Rothamsted experiments illustrate this in a very striking manner. The following table gives the yield of wheat from plats 3, 11, 12, 13, and 14:

TABLE 4

Average yearly yield of wheat for 51 years on the Rothamsted Experimental Farm

PLAT NO.	TREATMENT	AVERAGE 40 YEARS 1852–92	1899	1900	1905
		bu.	*bu.*	*bu.*	*bu.*
3	Unmanured continuously	12⅞	12	12¼	18.0
11	400 lbs. ammonium salts, 350 lbs. superphosphates	24¼	21¼	18¼	18.9
12	400 lbs. ammonium salts, 350 lbs. superphosphates, 366½ lbs. sodium sulfate	30	28¾	24¾	30.5
13	400 lbs. ammonium salts, 350 lbs. superphosphates, 200 lbs. potassium sulfate	31½	26¾	28⅛	39.4
14	400 lbs. ammonium salts, 350 lbs. superphosphates, 280 lbs. magnesium sulfate	30⅜	28¼	23¼	26.0

It may be seen from table 4 that the plat which received sodium sulfate gave as an average 30 bushels per acre, or 5½ bushels more than plat 11 which, with the exception of the sodium sulfate, was treated the same. This yield is within 1½ bushels of that of plat 13 which received the potassium sulfate. This beneficial effect produced by sodium sulfate is usually attributed to the liberation of potassium. While a considerable part of this beneficial effect is undoubtedly due to this cause, a study of the phosphorus yielded by each plat at least indicates that there is another factor entering. The average yield of phosphorus from plat 11 was 8.27 pounds (62) per acre; while the average on plat 12 was 9.82 pounds per acre. It may thus be seen that as an average of 20 years there were 1.55 pounds more of phosphorus taken from the sulfate plat than from the plat which received no sulfate. When one takes into consideration this excess of 31 pounds of phosphorus which had been removed it would seem that the sulfate

had in some way made the phosphorus more available. As an average of 40 years the plat which received superphosphate alone had 16.46 per cent (34) phosphorus in the ash of the wheat. The plat which received sodium, potassium, and magnesium salts in addition to the superphosphate had 16.78 per cent phosphorus in the ash of the wheat.

The above facts point very strongly to a liberation of phosphorus by various sulfates. This is well shown by the work of Dyer (21). He made a careful study of the soil from the Hoos Field, Rothamsted. The land had been in barley for 42 years. The plat which had received no manure was found to contain 22.27 pounds more phosphorus per acre in the first nine inches than the one which had received ammonium salts. However, the amount soluble in a 1 per cent solution of citric acid was 5.24 pounds more in the latter than in the former. When the plats throughout the entire field were taken the same relationship was found to hold. There was more soluble phosphorus in every case in the plats which had received a sodium, potassium, magnesium, or ammonium salt. If we take Dyer's averages of the plats which were treated nearly alike this fact is brought out even more forcibly than the above. The four plats which received nitrogen but no mineral yielded, as an average of 38 years, $28\frac{1}{2}$ bushels of barley per acre. The soluble phosphorus in these plats was 69 pounds per acre. Now, taking the four plats which received nitrogen, sodium, potassium, and magnesium but no phosphoric acid, they yielded, as an average for the same length of time, $20\frac{1}{2}$ bushels, and contained 103.47 pounds per acre of soluble phosphorus. It may be seen that the latter in the course of 38 years yielded 72 bushels more barley than the former and at the end of this period had 32.61 pounds per acre more soluble phosphorus in the soil. Again, the four plats which received a complete fertilizer had an average yearly yield of $39\frac{3}{8}$ bushels per acre. The plats which received only nitrogen and phosphorus yielded $38\frac{7}{8}$ bushels. At the end of the period there were 549.7 pounds of soluble phosphorus in the one which received a complete fertilizer, while the plat which received nitrogen and phosphorus had only 477.6 pounds of soluble phosphorus per acre in the first 9 inches. This is 72.1 pounds in favor of the plats which received sulfates.

Later Dyer (21a) made a study of the Rothamsted wheat soil, determining the potassium and phosphorus present in a soluble and insoluble condition, and found that pound for pound the magnesium salts are the most effective in keeping the phosphorus soluble. The sodium sulfate comes next and the potassium salts have least effect.

Manganese compounds

Manganese is looked upon as one of the most active catalizers, but the results obtained with it are not always concordant (100, 112).

Some experiments by Skinner and Sullivan (128) demonstrated the fact that manganese acts in various ways as a fertilizer. It is often without influence,

occasionally injurious, but usually beneficial, its effect depending apparently upon the composition and character of the soil. The oxidation in soils under treatment with manganese salts was also studied and it was found that an increase in oxidation and growth frequently occurred in aqueous extracts of poor, unproductive soils; but while oxidation was increased in fertile soils, growth was decreased, the plants showing indications of excessive oxidation. Field experiments showed practically no effect from the manganese salts, but the soil was acid, a condition which may have accounted to a considerable degree for the nature of the results.

It is suggested that when the action of manganese is beneficial.

> It is probably due (a) to the increased oxidation produced in the plant roots whereby the plant is stimulated to greater activity and to increased absorption of the material useful for its growth and general metabolism; (b) *to the stimulation of the activity of microörganisms in the soil*; (*c*) to an increased oxidation within the soil.

The same authors also suggest that when large applications of manganese have been found to be injurious, the injury is undoubtedly due to the "excessive stimulation and excessive oxidation in microörganisms and in the plant, with a resulting charge in the biochemical activities of plant and microörganisms and in the conditions of inorganic and organic soil constituents, the ultimate result of which change is injurious to the growing crop."

An Italian investigator (19) found that manganese carbonate added to the soil with a natural phosphocarbonate greatly increased the yield of wheat and alfalfa, whereas Pfeiffer and Blanck (100) found that manganese caused an increased assimilation of nutritive substances from the soil.

Although the addition of manganese to a soil often increases the nitrogen content of the crop (125) it does not increase the manganese content (44), thus indicating that its action is mainly on the biological transformation of nitrogen and possibly phosphorus which is going on in the soil.

Effect of saline solutions on phosphates

A number of experimenters have done some work in the laboratory to determine the solvent action of various salts in solution on phosphates.

The following is a brief summary of the most important work done on this subject:

Schulov (116) found that a solution of ammonium sulfate extracted more phosphorus from a phosphorite than did the same volume of distilled water. This was found to be true with the nitrate also, but not to as great an extent as with the sulfate solution.

Cameron (14), working with chemically pure iron phosphate, aluminum phosphate, and calcium phosphate, found that the calcium phosphate was slightly more soluble in a solution of potassium chlorid, and less soluble in calcium chloride and calcium nitrate than in distilled water. The iron phos-

phate was more soluble in a potassium sulfate solution and less soluble in a potassium chloride and a sodium nitrate solution than in water. He showed further that equilibrium was not established until at least ten days after the solution had been added to the phosphate.

Kalmann and Bocker (49) extracted soil with a solution of calcium sulfate and with distilled water and obtained the same amount of phosphorus in each case.

Fedler (27) obtained less phosphorus by extracting the soil with sodium nitrate solution than with distilled water. Krouch (60), however, obtained more with a sodium chloride solution than with water. Thompson (143) found the same to hold true when a superphosphate was used in the place of soil.

Both Kellner (55) and Sutherst (142) found that lime rendered the phosphorus of the soil more soluble. Later, Hartwell and Kellogg (42), at the Rhode Island Station, found the same to be true.

Voelcker (152), working with phosphate, bone meal, Cambridge and Suffolk coprolites, found that they were all more soluble in ammonium chloride and in ammonium carbonate solution than in distilled water. Sodium nitrate solution extracted more of the phosphate than did water, while the coprolites yielded the same to each solvent.

Sutherst (142) found that lime extracted more phosphorus from iron and aluminum phosphates than water, but when calcium carbonate was used in place of the lime the same amount of phosphorus was obtained with each solvent. He also found that potassium chloride and sodium chloride solutions each extracted less from bone meal than did distilled water. However, when he used bone flour in place of bone meal, each salt extracted considerably more than the distilled water. He explains this apparent contradiction by assuming that bone meal, when in the soil, undergoes fermentation by which the phosphorus is rendered more soluble, but when quantities of inorganic salts are present this fermentation is prevented.

Liebig (65) showed that sodium nitrate increased the solubility of calcium phosphate, while Lachonicy found that it decreased the solubility of iron phosphate.

Guthrie and Cohen (41) found the amount of water-soluble plant-food larger in limed than in unlimed soil, but only in a sandy soil did liming increase the proportion of water-soluble phosphorus and potassium over that orginally present in the soil. This is due to the calcium of the lime replacing the iron and aluminum which is in combination with the phosphorus of the soil (32). Calcium carbonate may have a similar action on soils rich in aluminum and iron phosphate (33).

König (57), however, found the phosphorus in a soil to be less available the greater the content of lime, magnesia, iron oxid, and clay in the soil.

Foster and Neville (29) found that with increasing quantities of ammonia the quantity of phosphorus passing into solution from various difficultly-soluble phosphates increased at first rapidly and then more slowly, the solubility

of the phosphate being almost directly proportional to the amount of ammonia present. This is similar to the results of Mitscherlich (84) who found the solubility of dicalcium and tricalcium phosphate in water saturated with carbon dioxid was greatly increased by the addition of ammonium sulfate and chloride and chloride and sulfate of sodium and magnesium. Aita (1) found that ammonium, alkali, and magnesium salts brought about an increase in solubility of phosphates proportional to their concentration and dependent upon the factors of friability, fineness, and calcium oxid content. The increase of solubility was a function of the anion.

Patten (93) concluded from his work that a higher concentration of phosphorus may be obtained when weak solutions of salts ordinarily used as soil amendments—potassium chloride, potassium sulfate, potassium and sodium nitrate, or potassium carbonate—are passed through the soil than can be obtained by the use of distilled water.

The senior author (37) tested the solubility of a number of different phosphates in a 1 per cent solution of the various salts with the results reported in table 5.

TABLE 5

Average of phosphorus dissolved by 450 cc. of a 1 per cent solution of each of the following solvents from 2 gm. of each of the phosphates

SOLVENT USED	PHOSPHORUS			
	Weathered phosphate	Unweathered phosphate	White phosphate	Soil and phosphate
	mgm.	*mgm.*	*mgm.*	*mgm.*
Distilled water	0.37	0.23	0.37	0.77
Ammonium nitrate	0.96	0.53	1.16	1.45
Ammonium sulfate	0.60	0.26	0.93	0.82
Sodium sulfate	0.55	0.17	0.82	0.97
Ammonium chloride	0.53	0.35	0.85	0.59
Potassium sulfate	0.51	0.25	0.73	0.95
Magnesium sulfate	0.51	0.28	0.69	0.69
Magnesium nitrate	0.47	0.33	0.70	1.25
Sodium nitrate	0.37	0.20	0.78	0.96
Magnesium chloride	0.37	0.25	0.62	0.68
Potassium chloride	0.35	0.15	0.56	0.43
Potassium nitrate	0.35	0.16	0.65	0.81
Sodium chloride	0.32	0.16	0.39	0.77
Calcium nitrate	0.28	0.22	0.15	0.45
Gypsum	0.20	0.20	0.25	
Calcium sulfate, c.p.	0.18	0.15	0.20	0.40
Calcium chloride	0.13	0.10	0.19	0.42
Iron sulfate	0.14	0.04	0.14	0.30

In every case the iron solution extracted less than the water. This would be expected, for if there were any exchange in the basic ions, there would result iron phosphate which is still less soluble in water than calcium phosphate.

In every case the potassium sulfate, ammonium sulfate, magnesium and ammonium nitrate extracted more phosphorus than did water. Disregarding the result obtained with the soil and phosphate mixed, we find that magnesium sulfate and ammonium chloride rendered the phosphate more soluble. This, however, is reversed on addition of soil to the phosphate. Sodium sulfate, except with the unweathered phosphate, extracted more than water. Comparing the results with sodium and potassium nitrate we find where the brown phosphate was used these solvents depressed the solubility, where the white phosphate (a phosphate containing more calcium and little iron) was used they rendered the phosphate more soluble. This is in accord with the work of other experimenters who have found that these salts depress the solubility of iron phosphate and increase the solubility of calcium phosphate. Sodium, magnesium, and potassium chloride have little if any effect on the phosphate.

Carbon dioxide on soluble plant-food of soil

Under some conditions the large quantities of carbon dioxide liberated from the rapidly decomposing fresh manure may be valuable in rendering soluble plant-food. Bornemann (5) found that soil constantly supplied with carbon dioxide through a pipe buried in the ground gave an increase in yield of 12.2 per cent over the crop grown on untreated soil. Wollny (164) has shown that manure greatly increases the carbon-dioxide production in a soil, and Truog (146) found that the addition of manure to a soil greatly increased the carbon-dioxide produced and for a short time measurably increased the solvent action on floats.

The quantity of carbon dioxide produced in a good aerable soil in 24 hours is enormous. Stoklasa (136) estimated from various determinations that there may be as much as 66.75 pounds per acre to a depth of 15.76 inches produced per day. The bacterial activity of a soil is measured by the amount of carbon dioxide formed in a soil in unit time and this varies widely with the physical and chemical conditions of the soil. Any factor which increases bacterial activity also increases the carbon dioxide generated.

Patten and Brown (92) decided that the carbon dioxide liberated by bacteria played an important part in the dissolving of phosphorus from difficultly soluble phosphates. Pfeiffer and Blanck (99) noted that the keeping of soil charged with carbon dioxide increased the assimilation of phosphorus from such a soil by plants. Although carbon dioxide increases the assimilation of phosphorus the quantity was often greater than could be accounted for by the solvent action of the carbon dioxide, thus indicating other factors at work (102). Herke (45) found sodium nitrate and potassium sulfate to increase the carbon dioxide generated in a soil.

Peterson (97) and Wollny (165) found that lime increased the carbon dioxide given off from soils, and Ebermeyer (22), Hilgard (46) and Hartwell and Kellogg (42) proved conclusively that lime increases the decay taking place in a soil.

Stimulation of bacteria by salts

Withers and Fraps (163) found that calcium carbonate added to a soil greatly accelerated nitrification and that it is especially desirable that it should be added where ammonium sulfate is being used as a fertilizer. Lipman's work (74) showed that calcium carbonate stimulated nitrification more than did gypsum, and that sodium chloride was injurious to nitrifying organisms, while ferrous sulfate in amounts from 10 to 100 mgm., per 100 gm., of soil was without effect. Later, he and Brown (77) decided that both ammonification and nitrification were promoted by magnesia lime to a more marked extent than they were by non-magnesia lime. This, however, was to a certain extent dependent upon the treatment and crop growing on the soil. Both ammonification and nitrification were accelerated by sodium nitrate. In a more recent work Lipman, Brown, and Owen (78) found that small applications of calcium carbonate stimulated bacterial activity, but large applications had detrimental effect upon ammonification.

In Owen's experiments (90), magnesium carbonate was more efficient in promoting ammonification and nitrification than was either calcium or potassium carbonate. According to Engberding (24) ammonium sulfate, sodium nitrate, potassium nitrate, and caustic lime all increase the bacterial content of the soil, but decrease its nitrogen-fixing powers. Krüger's work (61) indicated that calcium carbonate was more effective in promoting nitrification than was lime, the reverse being true with regard to the putrefactive bacteria. The formation of ammonia from peptone was especially favored by calcium carbonate. Lyon and Bizzel (81) found that lime favored nitrification, as did also certain nodule-bearing legumes. Fischer (28) concluded that the presence of calcium carbonate in a nutritive solution favored the formation of protein nitrogen, while magnesium carbonate lessened the transformation of ammonia into protein nitrogen. Calcium oxide, however, exerted a much greater influence upon soil bacteria than did calcium carbonate.

Peck (95) studied the influence of a number of salts upon bacterial activity when applied to the soil, with the result that the carbonate, sulfate and phosphate of calcium were found to stimulate ammonification, while sodium nitrate depressed it; both potassium sulfate and calcium carbonate accelerated nitrification in soil. Brown (10), working with a typical Wisconsin drift soil, found that the application of ground lime up to 3 tons per acre increased the number of bacteria in the soil, also the ammonifying, nitrifying, and nitrogen-fixing powers of the soil. The increase was, in every case, nearly proportional to the limestone applied.

At the times the increase noted in ammonification is due to the retention of the volatile ammonia by the carbonate, as is shown by Lemmermann's results (64) where the addition of calcium carbonate to a soil up to 1 per cent reduced the volatilization of ammonia, but calcium oxide had the opposite effect. Both calcium chloride and calcium sulfate reduced the loss of ammonia, but the chlor-

ide was the only salt of magnesium tested which had this effect. Potassium and sodium chlorides, sulfates and carbonates all reduced the absorptive powers of the soil. Paterson (91) studied the influence of a number of substances upon nitrification with the result that caustic lime was found practically to stop all nitrification. Calcium carbonate promoted it, as did also magnesium carbonate; gypsum was less effective, while ferric hydrate had a very favorable effect.

Kelley (52) studied the effect of calcium and magnesium carbonate alone and in combination upon ammonification and nitrification. In his work calcium carbonate only slightly stimulated ammonification of dried blood, but it had a marked stimulating effect upon nitrification. The magnesium carbonate was found to be toxic to both groups of organisms. No antagonism was found to exist between calcium and magnesium. Later, when working with Hawaiian soils, he (53) reports a stimulation for both. The results, however, varied with different soils, and he considers the lime-magnesia ratio of little importance as regards the ammonifying and nitrifying organisms. Allen's conclusion (2) is that large quantities of limestone must be applied to a non-calcareous soil in order to bring its nitrifying powers up to those of natural calcareous soils.

Chester (15, 16) showed that lime increases the number of bacteria in soil, the increase being proportional to the lime applied up to 4,000 pounds per acre. He considered the effect as due to the lime giving to the soil a more favorable reaction for the growth of bacteria and not to its direct action upon the organisms themselves.

These findings were later confirmed by Fabricicus and von Feilitzen (26), Engberding (24), Ehrenberg (23), and Fischer (28).

Lime not only increases the number of organisms in a soil, but it increases the ammonifying powers of the soil, as is seen from the work of Remy (110), Ehrenberg (23), and Voorhees and Lipman (155).

The literature dealing with the influence of lime upon the nitrifying organisms is voluminous. No attempt is made here to refer to all of it, for in most cases the experiments were conducted on soils which were acid and the lime supplied neutralized the acidity of the soil, thus giving the necessary neutral reaction for the action of the nitrifying organisms. Such results give little, if any, idea of the direct stimulating influence of calcium. Furthermore, the work has recently been summarized by Brown (10) who concludes that the application of lime increased nitrate production from ammonium sulfate and dried blood, the gain being almost proportional to the quantity of lime applied. This, in turn, was found to bear a close relationship to the number of organisms developing on synthetic agar.

Severin (122) concludes from his work that gypsum not only prevents the loss of ammonia from manure, but it increases the speed of decomposition from 10 to 20 per cent, while Paterson and Scott (91) state that gypsum slightly increases nitrification in soil as determined by laboratory experiments. Prior to this, Pichard (103) had shown that the sulfates of calcium, potassium, and sodium promote nitrification.

Opposite results are reported by Dezani (18) who found that gypsum in amounts varying from 0.5 to 2 per cent had no appreciable effect on nitrification, while the results obtained by Lipman and others (76) varied and were inconclusive.

According to Lipman (66) calcium chloride in solutions accelerated the action of ammonifiers, and it is interesting to note that in a later work he (67) failed to find antagonism between either calcium and magnesium or calcium and sodium. The chlorides of calcium, magnesium, potassium, and sodium were found to be toxic in the order named. Marked antagonism exists between calcium and potassium, magnesium and sodium, and potassium and sodium. Sea water was found to be a physiologically balanced solution for *Bacillus subtilis*.

According to Paterson and Scott (91) ferric hydrate has a distinctly beneficial effect upon nitrification. In this latter case its action could be due to its serving as a base. According to Lipman and Burgess (71) the ammonifiers are more sensitive to iron sulfate than are the nitrifiers, for while the latter were stimlated by small amounts of iron sulfate, it was toxic to the former in all concentrations tested.

Magnesium compounds usually stimulate bacterial activities to a greater extent than do calcium compounds, as had been noted in some of the literature already cited, but Engberding's results (24) showed that while magnesium sulfate stimulated bacterial activities, it was not as effective in this regard as was ammonium sulfate, sodium nitrate, or potassium sulfate. The work of Makrinov (82) is of interest, as he found pure maganesium carbonate a very suitable substance on which to grow the nitrous organism. Furthermore, magnesium carbonate had a very beneficial effect on the physiological action of the organism. Kellerman and Robinson (51), on the other hand, found that magnesium carbonate when applied to a soil already rich in magnesium carbonate positively inhibited nitrification if the quantity added exceeded 0.25 per cent. We have here an apparent contradiction, but it may be due to the different conditions of the experiments, as one investigator was working with cultures of the organisms while the other was using the soil with its complex flora. Furthermore, it is quite possible that magnesium carbonate may be without effect upon or even accelerate the growth and activity of the nitrosomonas and yet inhibit the nitromons.

Manganese stimulates higher plants and in many cases acts as a positive catalyzer. Skinner and Sullivan (128) conclude from some of their experiments that manganese salts increase the oxidation in some soils. But while Montanan (85) found that manganese carbonate, sulfate, and dioxid greatly stimulated nitrification, he attributed it to either the direct or the indirect furnishing of oxygen to the nitrifying organisms, and not to any catalytic effect of the manganese itself. Beijerinck (4) observes that some soil organisms have the power of oxidizing manganous carbonate. Olaru (89) found that manganese in the proportion of 1 part to 200,000 parts of nutritive media greatly increases

nitrogen fixation, and he considers it quite likely that the increased yield obtained after the application of manganese compounds to a soil is due to its accelerating the action of the nitrogen-fixing organisms of the soil.

Potassium is essential for the nutrition of both the higher and lower forms of plant life. Hence, it is to be expected that when added to a medium poor in potassium it will increase bacterial growth, but like many other true nutrients it may become toxic if present in too large a concentration. As already noted, Engberding (24) states that potassium sulfate caused a slight increase in the bacterial content of a soil. While Peck (95) found it to increase nitrification in soils, Renault (111) claims that slow ammonification and subsequent nitrification is always accompanied by a low percentage of potash. Dumont's (20) experiments showed that potassium carbonate, added to a soil at the rate of from 1 to 2.5 gm. per 1000 gm. of soil, markedly increased nitrification, but that larger applications of the salt progressively diminished the rate of nitrification, while the addition of 8 gm. to 1000 gm. of soil completely checked it. Lumia (80) concluded that potassium chloride and sulfate were nearly as effective in promoting the activity of alcoholic ferments as were phosphates.

Fred and Hart (31) found that both calcium and potassium sulfates increased ammonification in solution, and that the sulfates of potassium, calcium, and magnesium each increased the evolution of carbon dioxide from soil. But from the results obtained with different salts, they conclude that the addition of the potassium ion did not materially increase ammonification in the soil examined.

Lipman (78) and others found that sodium nitrate increased the accumulation of nitrates in a soil. They found, however, a certain periodicity in the accumulation of nitrates which would account for the different results reported by various investigators, and in later investigations they (76) concluded that at times sodium nitrate stimulates ammonification.

C. B. Lipman (68) demonstrated that ammonification is inhibited by sodium chloride, sodium sulfate, and sodium carbonate. The points at which the salts become toxic are: for sodium chloride, between 0.1 per cent and 0.2 per cent; for sodium sulfate, 0.4 per cent; and for sodium carbonate, 2.0 per cent. A stimulating influence was noted in the case of sodium carbonate, but not in the case of the sulfate or of the chloride. The points at which they became toxic to nitrifiers (69) were found to be: for sodium carbonate, 0.025 per cent; for sodium sulfate, 0.35 per cent; and for sodium chloride, less than 0.1 per cent.

All except carbonate acted as a stimulant in lower concentrations. Later Lipman and Sharp (73) found the point at which sodium chloride became toxic to nitrogen-fixing organisms in soil to be from 0.5 to 0.6 per cent; sodium sulfate, at 1.25 per cent; and sodium carbonate, at 0.4 to 0.5 per cent. Sodium chloride was the only one which acted as a stimulant.

A very extensive piece of work by the authors (39) revealed the fact that sodium carbonate, nitrate, and chloride, potassium carbonate and nitrate, calcium carbonate and sulfate, magnesium sulfate and carbonate, iron nitrate

sulfate carbonate and chloride, and all of the salts of manganese stimulated ammonification. Sodium nitrate and chloride, potassium nitrate and chloride, calcium nitrate, chloride and sulfate, magnesium nitrate, chloride and carbonate, iron sulfate, carbonate, and chloride, all of the salts of manganese stimulated nitrification. The extent of the stimulation varied with the specific salt, as may be seen in fig. 1 wherein the untreated soil is taken as 100 per cent.

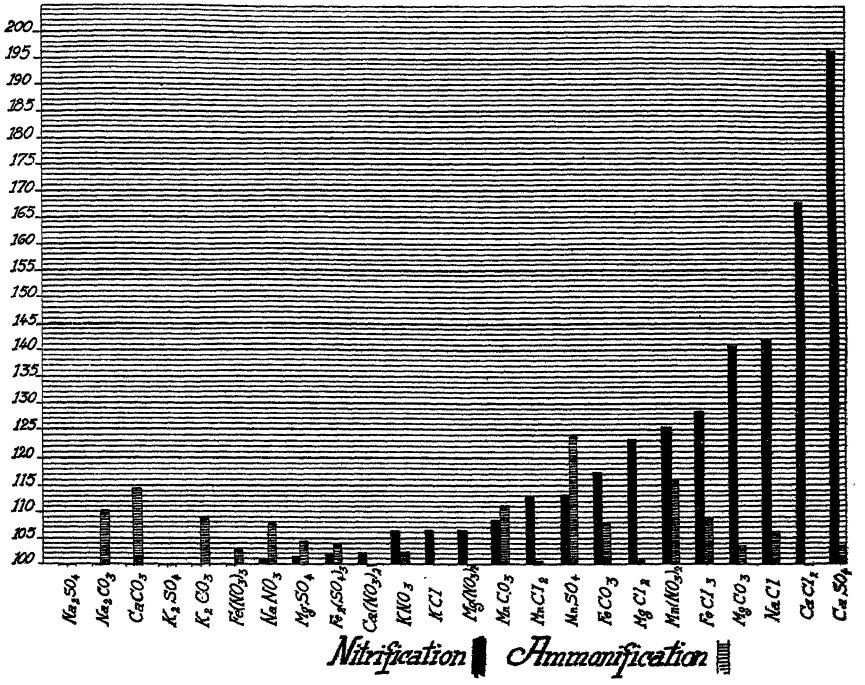

FIG. 1. DIAGRAM SHOWING PER CENT OF AMMONIA AND NITRIC NITROGEN PRODUCED BY BACTERIA IN SOIL RECEIVING VARIOUS SALTS, THE UNTREATED SOIL CONSIDERED AS 100 PER CENT

It is very significant that calcium sulfate, calcium chloride, sodium chloride, and the compounds of iron and manganese, which we have found such marked plant stimulants, are also the most active bacterial stimulants. The results point to the conclusion that the stimulation of the plant is due in no small degree to the increased nitrogen and phosphorus due to the increased bacterial activity of the soil.

Solvent action of bacteria

Brown (9) found that 12 out of the 23 bacteria isolated from soil exerted a definite solvent action on difficultly soluble plant-food. One organism which produced no gas but a large amount of acid showed the greatest solvent action

upon calcium carbonate, while other organisms which produced gas—largely carbon dioxide—but not as much acid as the former gave an action more marked than of the stronger acid-producer upon the dicalcium and tricalcium phosphates. *Bacillus subtilis, Bacillus mycoides, Bacillus proteus vulgaris, and Bacillus coli communis,* as well as several agar cultures from garden soil, were found (114) to be capable of dissolving the phosphates of bone and to a less extent that of mineral phosphates. The greatest solvent action was exerted in media containing sodium chloride, potassium sulfate, and ferrous sulfate. Even yeast (58) may play an important part in dissolving phosphates. But Khober considers that the life activity of the bacteria, that is, assimilation of phosphorus by the living organism, plays little or no direct part in solution of the phosphates, but that the latter is due to the action of the organic acid and of the carbon dioxide produced.

The acids produced by bacteria act upon all kinds of phosphates changing them to the soluble monophosphate, but the rate of solution varies widely with the different phosphates. Tricalcium phosphate, in precipitated form, dicalcium phosphate, and tetracalcium phosphate of Thomas slag are much more rapidly dissolved than the crystalline or the so-called amorphous phosphates. The general reaction is as follows:

$$2\ RCOOH + Ca_3(PO_4)_2 \rightarrow Ca_2H_2(PO_4)_2 + (RCOO)_2Ca$$

The reaction takes place most rapidly in soils containg large quantities of organic matter due to the active fermentation taking place in such soils.

Grazia (36) considers enzyme action to play a part in the dissolving of phosphates in soil, for he found the addition of chlorofrom to a soil reduced bacterial activity and decreased the acid produced, but at the same time the solution of phosphates was increased. This is in keeping with the finding of Bychikhin and Skalski (13).

The presence of ammonium chloride and sulfate in the cultural media is especially effective, according to Perotti (96) in increasing the solvent action of bacteria, for phosphorus. Perotti considers the successive steps in the solution or decomposition of phosphorus in bacteria cultures as follows: (a) generation of acids; (b) secondary reactions in the solution; and (c) production of a soluble phosphorus-containing organic substance. The first two of these are the result of the activity of the bacteria on the phosphorus, and the last is due to the metabolic assimilation of the microörganisms.

The oxidation of sulfur by soil bacteria may at times generate sufficient acid to play a very important rôle in the dissolving of soil phosphorus. However Hopkins and Whiting (47) consider that the nitrite bacteria are of first importance in rendering phosphorus and calcium soluble due to the nitrous acid generated from the ammonia.

$$(NH_4)_2\,CO_3 + 6\,O = 2\,HNO_2 + H_2CO_3 + 2\,H_2O$$

The resulting nitrous acid reacts with the raw rock phosphate rendering it soluble, thus:

$$Ca_3(PO_4)_2 + 4\ HNO_2 = CaH_4(PO_4)_2 + 2Ca(NO_2)_2$$

The actual ratio found showed that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized aside from the action of the acid radicles associated with the ammonia. The carbonic acid would play an important part also in this reaction:

$$4H_2CO_3 + Ca_3(PO_4)_2 = 2\ Ca(HCO_3)_2 + CaH_4(PO_4)_2$$

They found that neither ammonia-producing bacteria nor nitrate bacteria liberated appreciable quantities of soluble phosphorus from insoluble phosphates.

Whereas this would readily occur in soil poor in calcium carbonate, yet in those rich in calcium carbonate there would be only small quantities of phosphorus liberated, is the conclusion reached by Kelley (53). But where the soluble phosphorus is rapidly being removed by the growing plant there is little doubt but that the various soil organisms play an important part in rendering phosphorus soluble.

Moreover, it is quite evident that *Azotobacter* in their metabolism transform soluble inorganic soil constituents either into soluble or into insoluble organic forms. This is especially true of phosphorus which is found in the ash of these organisms in such large quantities. The phosphorus, on the death of the organism, is returned to the soil in a readily available form, for Stoklasa has found that 50 per cent of the nitrogen of these organisms is nitrified within six weeks, and there is no reason for believing that the phosphorus would be liberated more slowly. Then there is the possibility that many of the constituents of the bacterial cell may become available through the action of autolytic enzymes without the intervention of other bacteria (79).

It is further evident that an organism which possesses the power when growing under appropriate conditions of generating 1.3 times its own body weight of carbon dioxide during 24 hours (135) must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent and will attack even ordinary quartz rock. Granite and rocks related to it are rather quickly attacked with the liberation of potassium and other elements. Likwise, it would act upon the tricalcium phosphate of the soil with the formation of more readily soluble phosphates, for this substance is four times as soluble in water charged with carbon dioxide as it is in pure water:

$$Ca_3(PO_4)_2 + 2\ CO_2 + 2\ H_2O = Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$$

Moreover, the nitrogen-fixing organisms form, among other products, formic, acetic, lactic, butyric, and other acids. The kind and quantity of each depends upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble

plant-food which may be rendered soluble, for they have a high solvent power for the insoluble phosphates (133). The resulting salts of calcium would be further attacked by bacteria with the formation of calcium carbonate (35).

Whether these processes will give rise to an increase in the water-soluble plant-food of the soil will depend upon whether the products of the second, the analytic reactions, exceed the products of the first, the synthetic reactions. We must not lose sight of the fact that, although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they were at first derived through bacterial activity.

This being the case, we may expect to find variations in the results reported from laboratory tests. Stoklasa (134) found that bacterial activity rendered the phosphorus of the soil more soluble, whereas Severin (123) in his early work found the opposite to be true. Others have found that the solvent action of bacteria for insoluble phosphates is in direct proportion to the acid secreted by the organism (114).

In a later work, Severin (124) obtained different results. He used three soils, one sterile, a second sterilized and inoculated with pure cultures of *Azotobacter*, and a third sterilized and inoculated with cultures of *B. radicicola* and *Azotobacter*. The solubility of the phosphorus increased 8 to 14 per cent over that in the sterile soil. The acid-producing organisms, due to the acid secreted and their intimate contact with the soil particles, possess the power of dissolving silicates (3). Moreover, arsenic greatly stimulates nitrogen-fixation and there is a relationship between this increased bacterial activity and the form and quantity of phosphorus found in such a soil (38).

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, yet these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers (136).

There are, however, cases in which bacterial activity has decreased the water-soluble phosphorus of the soil and of raw rock phosphate (144, 145). But this does not mean that it is less available, for as pointed out by Truog (146) the mixing of floats with manure caused an immediate decrease in the solubility of the phosphorus in 0.2 per cent citic acid solution, yet when thoroughly mixed with the feeding area of the soil its availability was increased to such an extent that some species of plants were apparently able to secure almost an adequate supply of phosphorus from this material. The addition of manure to the soil greatly increased the carbon-dioxid production and for a short time measurably increased the solvent action on floats. Where there is for a time a decrease of water-soluble phosphorus in fermenting media it is probably due to the formation of phosphoproteids within the bodies of the bacteria (127), and these would later be rendered soluble due either to further bacterial activity or autolytic enzymes.

Action of salts and bacteria on phosphorus

We have shown that many salts when applied to a soil increases the accumulation of ammonia and nitric nitrogen in such a soil (39). The magnitude of this stimulation varies with the specific salt and the quantity added. The quantity necessary for maximum stimulation of ammonification and nitrification is given in figure 2, whereas in figure 1 is given the extent of this stimulation.

The question naturally arises as to what effect, if any, this increased stimulation is going to have upon the phosphorus of the soil. We have attempted to answer this question by the following: To tumblers containing 100 gm. of soil and 2 gm., of dried blood were added the fraction of the gram mole which is indicated in the above graph as producing maximum nitrification. These were incubated for 21 days with a moisture content of 20 per cent, and at the end of this time the water-soluble and organic phosphorus were determined.

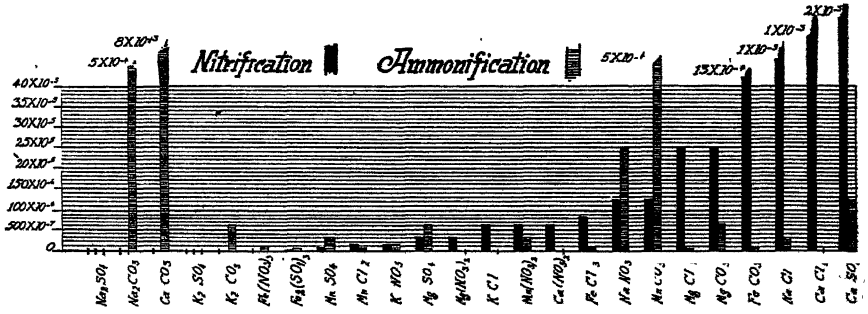

FIG. 2. DIAGRAM SHOWING FRACTION OF MOLECULAR CONCENTRATION OF VARIOUS SALTS IN 100 GRAMS OF SOIL NECESSARY TO PRODUCE MAXIMUM STIMULATION OF AMMONIFICATION AND NITRIFICATION

The water-soluble determinations were made by extracting with water as outlined in a previous article (37). The organic phosphorus determinations were made by the Schmoeger method which consists of extracting ignited and unignited soil with 12 per cent cold hydrochloric acid. In the case of the soil receiving no salt, 12 tumblers of soil were incubated and the phosphorus determinations made on each, and on the salt-treated soil 6 determinations were made. Therefore the results as reported are the average of either 6 or 12 separate determinations with the probable error calculated according to the formula:

$$E = \pm 0.7 \sqrt{\frac{S}{n(n-1)}}$$

S = the sum of the squares of the difference between each result and the average of all the determinations. N = the number of determinations in the average. The results for the sodium salts are given in table 6.

TABLE 6

Water-soluble and organic phosphorus in 100 gm. of soil receiving various sodium salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
None	5.05	100.0	±0.05	6.88	100.0	±0.22
5×10^{-4} mol. Na_2CO_3	5.92	117.2	±0.10	6.42	93.3	±0.43
1×10^{-3} mol. NaCl	5.52	109.3	±0.12	9.54	138.7	±0.69
125×10^{-6} mol. $NaNO_3$	5.10	101.0	±0.08	6.78	98.5	±0.30

All three of the sodium salts increased the water-soluble phosphorus of the soil. This is very marked in the case of sodium carbonate. In this case both the organic and inorganic phosphorus contributed to the water-soluble phosphorus. Sodium chloride when acting in conjunction with bacteria increased the water-soluble phosphorus of the soil 9.3 per cent. This is due to increased bacterial activity and not to direct solvent action of the sodium chloride, for this compound when first applied to a soil fails to increase its water-soluble phosphorus (37). In this case we must conclude that it and 38.7 per cent of the organic phosphorus must come from the more difficultly soluble mineral phosphorus of the soil. These results are a striking confirmation of the theory that the increased yield resulting from the application of sodium chloride to a soil is due in a large measure to its indirect action upon the phosphorus. True, there would also result an increased available supply of nitrogen to the plant, and it is certain that these two factors play a more important part than the direct stimulation of the plant by the sodium chloride. Moreover, it would appear that the active agent is the anion and not the cation.

The results obtained for the potassium salts are given in table 7.

TABLE 7

Water-soluble and organic phosphorus in 100 gm. of soil receiving various potassium salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
156×10^{-7} mol. KNO_3	5.46	108.1	±0.09	7.11	103.3	±0.24
625×10^{-7} mol. KCl	5.34	105.8	±0.17	7.38	107.3	±0.28
625×10^{-7} mol. K_2CO_3	4.71	93.3	±0.16	7.62	110.8	±0.35

Both the chloride and nitrate of potassium increased the water-soluble phosphorus of the soil. This is due to its action upon the soil microörganisms and not to its direct solvent action upon the soil phosphorus, for aqueous solutions of these salts dissolve no more phosphorus from raw rock phosphate or soil than do distilled water (37). Moreover, this increased soluble phosphorus must come from the insoluble phosphates and not from the organic phosphorus of the soil, for these two salts increase the organic phosphorus of the soil 7 and

3 per cent, respectively. The increase in the inorganic phosphorus resulting when potassium carbonate is added to the soil may come mainly from the water-soluble phosphorus, for there is nearly a corresponding decrease in the water-soluble.

The results obtained for the calcium salts are given in table 8.

TABLE 8

Water-soluble and organic phosphorus in 100 gm. of soil receiving various calcium salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
1 x 10^{-3} mol. $CaCl_2$	5.80	114.9	±0.06	6.07	88.2	±0.19
625 x 10^{-7} mol. $Ca(NO_3)_2$	5.77	114.3	±0.25	6.71	97.5	±0.42
8 x 10^{-3} mol. $CaCO_3$	4.25	102.2	±0.08	6.60	95.9	±0.34
2 x 10^{-3} mol. $CaSO_4$	3.71	73.5	±0.07	7.11	103.3	±0.25

Calcium chloride and calcium nitrate are about equally efficient in increasing the water-soluble phosphorus of the soil. But this is due to their action upon the bacteria, for, as would be expected, both of these compounds at first decrease the solubility of soil phosphorus (37). In the case of the calcium chloride the increased water-soluble phosphorus may come from the organic phosphorus of the soil, but in the case of the calcium nitrate it must come mainly from the insoluble mineral phosphates of the soil. Calcium carbonate increases the water-soluble phosphorus of the soil but decreases the organic phosphorus of the soil. This soil in the untreated condition contained over 12 per cent of calcium carbonate, and it may be that a soil poor in calcium carbonate would be stimulated to a much greater extent. Calcium sulfate is the only calcium compound that increases the organic phosphorus, and in this case it is only 3.3 per cent, whereas the decrease in the water-soluble phosphorus amounts to 16.5 per cent. It is therefore quite evident that increased yields resulting from the use of this compound are due mainly to its action upon the available nitrogen of the soil, for it increases the nitric nitrogen content of the soil 95 per cent (39), and only to a very small, if any, degree to the more available phosphorus.

The results obtained for the magnesium salts are given in table 9.

TABLE 9

Water-soluble and organic phosphorus in 100 gm. of soil receiving various magnesium salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
312 x 10^{-7} mol. $Mg(NO_3)_2$	5.83	115.5	±0.50	6.54	95.1	±0.34
25 x 10^{-5} mol. $MgCl_2$	5.54	109.7	±0.12	6.64	96.5	±0.19
312 x 10^{-7} mol. $MgSO_4$	5.31	105.2	±0.15	7.68	111.6	±0.53
25 x 10^{-5} mol. $MgCO_3$	4.96	98.2	±0.20	4.97	72.2	±0.06

All of the magnesium compounds except the carbonate increase materially the water-soluble phosphorus of the soil. With the magnesium nitrate and sulfate this may be due largely to the direct solvent action of the salt, for both of these compounds increase directly the solubility of phosphorus (37). But the chloride does not have this same effect. Hence, here it must be due to the action of the salt upon the bacterial flora of the soil. Much of the increased soluble phosphorus must have come from the insoluble mineral phosphates of the soil, for in none of the cases is the decrease in the organic phosphorus sufficient to account for the increase in water-soluble phosphorus. Although the magnesium sulfate increases the water-soluble phosphorus 5.2 per cent, yet the organic phosphorus is increased 11.6 per cent, making this the most efficient phosphorus solvent. This corresponds with its action upon plants and makes it certain that its indirect effect is due mainly to its action upon the insoluble phosphorus of the soil.

Magnesium carbonate had little effect upon the water-soluble phosphorus of the soil, but decreases the organic phosphorus 27.8 per cent. This would make it appear probable that the increased nitric nitrogen of the soil, which in this case amounts to 45.5 per cent over the untreated soil (39), must be due in a large measure to its decreasing the action of bacteria which feed upon nitrates in the soil. Otherwise, if there be an increased bacterial flora we would necessarily have found an increase in the organic phosphorus, for it is inconceivable to think of the organisms functioning without phosphorus in the nucleo-proteins which they must form.

The results for the manganese salts are given in table 10.

TABLE 10

Water-soluble and organic phosphorus in 100 gm. of soil receiving various manganese salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
625×10^{-7} mol. $MnCO_3$	5.43	107.5	±0.12	11.19	162.6	±0.35
156×10^{-7} mol. $MnCl_2$	5.06	100.2	±0.07	6.77	98.4	±0.16
78×10^{-7} mol. $MnSo_4$	4.76	94.3	±0.19	7.42	107.9	±0.57
625×10^{-7} mol. $Mn(NO_3)_2$	4.26	84.4	±0.37	6.05	87.8	±0.19

Manganese carbonate is the only one of the manganese compounds which increases the water-soluble phosphorus of the soil. This compound increases the organic phosphorus of the soil 62.6 per cent; hence, this offers a very plausible explanation of why greater yields are obtained when this compound is added to the soil with insoluble phosphate (19). It is not unlikely that much of the benefit resulting from the use of the sulfate is also due to its action upon the soil phosphate. These results, together with the increase in the available nitrogen supply of the soil (fig. 1), are ample to account for the increased yields noted when manganese compounds are applied to the soil.

The results for the iron salts are given in table 11.

TABLE 11

Water-soluble and organic phosphorus in 100 gm. of soil receiving various iron salts

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
104×10^{-7} mol. $Fe(NO_3)_3$	6.34	125.6	±0.05	5.58	81.1	±0.42
84×10^{-5} mol. $FeCl_3$	5.33	105.6	±0.13	6.52	94.8	±0.24
13×10^{-4} mol. $FeCO_3$	5.14	101.8	±0.45	5.72	83.1	±0.55
26×10^{-7} mol. $Fe_2(SO_4)_3$	4.76	94.3	±0.37	9.79	142.3	±0.42

Three of the iron salts, the nitrate, chloride, and carbonate, increase the water-soluble phosphorus of the soil. The nitrate increases the solubility 25.6 per cent, but 18.9 per cent of this may have come from the organic phosphorus of the soil, thus leaving the action on the insoluble mineral phosphates small. The increased water-soluble phosphorus resulting where the chloride and carbonate are used is small and may all come from the organic phosphorus of the soil.

Iron sulfate is one of the more powerful soil stimulants, and the field and pot experiments which have been cited indicate that it increases the phosphorus assimilated by the plant. This is not due directly to its solvent action upon the insoluble phosphorus, for at first it greatly decreased its solubility (37). However, these results show it to increase the organic phosphorus of the soil 42.3 per cent, 36.6 per cent of which must have come from the insoluble mineral phosphorus of the soil. It is very probable that this would rapidly become available to the growing plant and this, together with the increased available nitrogen, would be ample to account for the noted gain in crops resulting from its use.

So far in this discussion we have been comparing the action of compounds having the same electro-positive but a varying electro-negative ion. Hence, the results considered have given us an insight into the influence of the anions Cl, SO_4, NO_3, and CO_3 directly and indirectly upon the phosphorus of the soil. It is therefore important that the compounds be compared where the anion is a constant and the cation a variable. This is done for the chloride in table 12.

Probably all of the chlorides increase the water-soluble phosphorus, but only two out of the six increase the organic phosphorus. It is quite evident that both the anion and the cation influence the action of the bacteria upon the phosphorus of the soil, but the influence of the anion probably predominates, for we find all the chlorides increasing the water-soluble phosphorus and all but one of the sulfates decreasing it.

TABLE 12

Water-soluble and organic phosphorus in 100 gm. of soil receiving various chlorids

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
1×10^{-3} mol. $CaCl_2$	5.80	114.9	±0.06	6.64	96.5	±0.19
25×10^{-5} mol. $MgCl_2$	5.54	109.7	±0.12	6.07	88.2	±0.19
1×10^{-2} mol. NaCl	5.52	109.3	±0.12	9.54	138.7	±0.69
625×10^{-3} mol. KCl	5.34	105.8	±0.17	7.38	107.3	±0.28
84×10^{-5} mol. $FeCl_3$	5.33	105.6	±0.07	6.52	94.8	±0.16
156×10^{-7} mol. $MnCl_2$	5.06	100.2	±0.20	6.77	98.4	±0.06

TABLE 13

Water-soluble and organic phosphorus in 100 gm. of soil receiving various sulfates

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
312×10^{-7} mol. $MgSO_4$	5.31	105.2	±0.15	7.68	111.6	±0.53
78×10^{-7} mol. $MnSO_4$	4.76	94.3	±0.19	7.42	107.9	±0.57
26×10^{-7} mol. $Fe_2(SO_4)_3$	4.76	94.3	±0.37	9.79	142.3	±0.42
2×10^{-3} mol. $CaSO_4$	3.71	73.5	±0.08	7.11	103.3	±0.34

Every one of the sulfates greatly increases the organic phosphorus of the soil. The "physiological influence" which has been noted by so many experimenters must be due to action of these salts upon the organic phosphorus. Moreover, many experimenters have given to the sulfates first place when used with insoluble phosphates. This would indicate that the organic phosphorus, which is probably largely nucleo-phosphates, becomes available quite readily for plant use.

The results for the carbonates are given in table 14.

TABLE 14

Water-soluble phosphorus and organic phosphorus in 100 gm. of soil receiving various carbonates

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
5×10^{-4} mol. Na_2CO_3	5.92	117.3	±0.10	6.42	93.3	±0.41
125×10^{-6} mol. $MnCO_3$	5.43	107.5	±0.12	11.19	162.6	±0.35
13×10^{-4} mol. $FeCO_3$	5.33	105.6	±0.45	5.72	83.1	±0.55
25×10^{-5} mol. $MgCO_3$	4.96	98.2	±0.20	4.97	72.2	±0.06
625×10^{-7} mol. K_2CO_3	4.71	93.3	±0.09	7.62	110.8	±0.24
8×10^{-3} mol. $CaCO_3$	4.25	102.2	±0.08	6.60	95.9	±0.34

Four of the carbonates increase the water-soluble and three increase the organic phosphorus of the soil. Only one is without effect, the magnesium carbonate. This may be due to the fact that this soil before treatment contains 12 per cent of calcium carbonate and 8 per cent of magnesium carbonate. Therefore it is likely that the phosphorus of a soil low in these constituents would be influenced by the addition of calcium and magnesium carbonate.

The results for the nitrates are given in table 15.

TABLE 15

Water-soluble phosphorus and organic phosphorus in 100 gm. of soil reciving various nitrates

TREATMENT	WATER-SOLUBLE PHOSPHORUS	PER CENT WATER-SOLUBLE PHOSPHORUS	PROBABLE ERROR	ORGANIC PHOSPHORUS	PER CENT ORGANIC PHOSPHORUS	PROBABLE ERROR
	mgm.			*mgm.*		
None	5.05	100.0	±0.05	6.88	100.0	±0.22
104 x 10^{-7} mol. $Fe(NO_3)_3$	6.34	125 6	±0.05	5.58	81.1	±0.42
312 x 10^{-7} mol. $Mg(NO_3)_2$	5.83	115.5	±0.50	6.54	95.1	±0.34
625 x 10^{-7} mol. $Ca(NO_3)_2$	5.77	114.3	±0.24	6.71	97.5	±0.42
156 x 10^{-7} mol. KNO_3	5.46	108.1	±0.09	7.71	103.3	±0.24
125 x 10^{-6} mol. $NaNO_3$	5.10	101.0	±0.08	6.78	98.5	±0.30
625 x 10^{-7} mol. $Mn(NO_3)_2$	4.26	84.4	±0.37	6.05	87.9	±0.19

All but one of the nitrates tested increased the water-soluble phosphorus of the soil and in this regard it is more efficient than is the chloride. Only one potassium nitrate, increases the organic phosphorus. The decrease in organic phosphorus throughout this series may be taken to indicate that the increased water-soluble phosphorus is not due to bacterial activity, but the increase is greater than when the soil or raw rock phosphate is extracted with aqueous solutions of the nitrates. Thus we may consider that the solvent action is due to the nitrous, carbonic, or other acids generated by the bacteria which react with the insoluble mineral phosphates. We must not lose sight of the fact that Perotti (96) suggests—that there are three steps in the solvent action of bacteria, and in the third there is produced a soluble phosphorus containing organic substance and it may be that some of the water-soluble phosphorus is organic in nature.

SUMMARY

The literature cited in the early part of this paper clearly demonstrated that the sulfates of magnesium, calcium and iron, the chlorides of sodium, potassium magnesium and calcium, and the iron and manganese salts are especially efficient as soil stimulants. Furthermore, the evidence was strong that in the case of at least sodium chloride, iron sulfate, and the sulfates of most salts, the action was due to increased available phosphorus, whereas with other compounds it was increased available nitrogen. This theory is corrobated by the results given in table 16.

TABLE 16

Per cent nitric nitrogen water-soluble and organic phosphorus occurring in soil receiving various salts

TREATMENT	NITRIC NITROGEN	WATER-SOLUBLE PHOSPHORUS	ORGANIC PHOSPHORUS
	per cent	*per cent*	*per cent*
None	100.0	100.0	100.0
312×10^{-7} mol. $MgSO_4$	101.2	105.2	111.6
26×10^{-7} mol. $Fe_2(SO_4)_3$	102.0	94.3	142.3
625×10^{-7} mol. $Ca(NO_3)_2$	102.0	114.3	97.5
156×10^{-7} mol. KNO_3	106.4	108.1	103.3
625×10^{-7} mol. KCl	106.5	105.8	107.3
312×10^{-7} mol. $Mg(NO_3)_2$	106.5	115.5	95.1
125×10^{-6} mol. $MnCO_3$	108.4	107.5	162.6
156×10^{-7} mol. $MnCl_2$	112.9	100.2	98.4
78×10^{-7} mol. $MnSO_4$	113.2	94.3	107.9
13×10^{-4} mol. $FeCO_3$	117.4	105.6	94.8
25×10^{-5} mol. $MgCl_2$	123.2	109.7	96.5
625×10^{-7} mol. $Mn(NO_3)_2$	125.4	84.8	87.9
84×10^{-5} mol. $FeCl_3$	128.3	105.6	94.8
25×10^{-5} mol. $MgCO_3$	140.7	98.2	72.2
1×10^{-3} mol. NaCl	142.0	109.3	138.7
1×10^{-3} mol. $CaCl_2$	167.2	114.9	88.2
2×10^{-3} mol. $CaSO_4$	196.7	73.5	103.3

It is quite evident from these results that the increased available nitrogen and phosphorus is sufficient to account for the noted increase in crop yields resulting from the use of these soil amendments. For the increase in nitric nitrogen varies from 1 to 96.7 per cent, whereas the water-soluble phosphorus is increased in all but five of the seventeen salts listed. This increase varies from 0.2 per cent up to 15.5 per cent. Three of the salts which failed to increase the water-soluble phosphorus increases the organic phosphorus. Hence, the available phosphorus has been increased in all but two of the seventeen salts listed. The increase in organic phosphorus varies from 3.3 per cent with the calcium sulfate to 62.6 per cent with the manganese carbonate. The strong stimulant, sodium chloride, acts to a great extent by rendering phosphorus soluble, whereas the equally strong stimulant, calcium sulfate, acts by rendering available more nitrogen.

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BOTANICAL COMPOSITION OF A PERMANENT PASTURE AS INFLUENCED BY FERTILIZERS OF DIFFERENT COMPOSITIONS

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In the experiments under consideration the character of vegetation in a field under permanent pasture containing mixed herbage, as influenced by the fertilizer constituents, phosphate, nitrate, and potash, used singly and in combinations of various ratios, was ascertained. The growth produced each year was recorded and the increase or decrease of each species existing originally in the field was noted from time to time. This fertilizer experiment on grass was started in 1910 at the Pennsylvania Experiment Station at State College, Pennsylvania, the plots being located on a permanent pasture where the composition of the vegetation was chiefly Canada bluegrass, Kentucky bluegrass, and timothy, with relatively smaller amounts of white and red clover. The pasture was used for grazing for several years preceding the first application of fertilizers. Since that time the hay has been cut annually, its botanical composition noted and the total yield recorded. Some marked differences have been noted in the composition of vegetation from the differently fertilized plots.

The soil is of the Hagerstown Series with the surface soil chiefly loam and the sub-soil chiefly clay and silty clay loam. The soil may be described as a grayish brown to brown mellow soil, varying in depths on the plots from about 7 to 11 inches; the sub-soil is a yellowish brown to reddish brown clay to silty clay loam, passing at about 24 inches into a stiff, heavy, yellowish-red clay which extends to a depth of 3 feet or more. The sub-soil contains some disintegrated rock fragments. The material is derived from the weathering of limestone. The topography of the area is rolling, insuring good drainage. The field under experimentation had previously been for 20 to 30 years in a rotation of corn, oats, wheat, each one year, and clover and timothy one year. In 1906–1907 the field was in wheat when it was seeded to timothy in the fall and to clover in the spring. It was pastured in 1908 and in 1909. The practice had been to apply a moderate application of manure once in a rotation, usually on the sod for the corn crop, and acid phosphate for the wheat at the rate of about 200 pounds per acre. The part of the field in which this experiment is located was never limed.

PLAN OF THE EXPERIMENT

The experiment was run in duplicate, using the three principal fertilizer ingredients, acid phosphate, sodium nitrate and potassium chloride, singly, in combinations of two, and in combinations of three, the ratios varying in 10 per cent stages. The scope of the experiment is explained best by the use of the triangular diagram, which has been used extensively in nutrient culture solution experiments, and in fertilizer studies (2). The triangular diagram is shown in colors in plate 1. Each of the 66 circles represents a fertilizer mixture. The red represents acid phosphate, P_2O_5; the black, sodium nitrate, NH_3; and the white, potassium chloride, K_2O. Circle or plot 1 receives acid phosphate alone. Plot 56 potassium chloride alone and plot 66 sodium nitrate alone. The series from 1 to 56 represents mixtures of acid phosphate (red), and potassium chloride (white). Plot 2 has 90 per cent of P_2O_5 and 10 per cent of K_2O. Plot 46 has 10 per cent P_9O_5, and 90 per cent K_2O. Plot 16 has equal amounts of P_2O_5 and K_2O. Likewise the mixtures represented by the series from 56 to 66 are mixtures of potassium chloride (white) and sodium nitrate (black). Plot 61 has equal amounts of NH_3 and K_2O. The series from 1 to 66 represents mixtures of P_2O_5 and NH_3. Plot 21 has equal amounts of P_2O_5 and NH_3. Circles on the base line 56 to 66 represent mixtures containing no P_2O_5. The next tier of circles, namely 46 to 55, represents mixtures containing throughout 10 per cent P_2O_5, but varying amounts of the other two constituents. Similarly the series from 37 to 45 represents throughout 20 per cent mixtures of P_2O_5; series 29–36, 30 per cent mixtures of P_2O_5 and so on upward until 1, the apex of the triangle is reached, where the composition is 100 per cent P_2O_5. Similarly circles on the line 1–66 represent 0 per cent K_2O; series 2–65 represents 10 per cent K_2O, but varying amounts of P_2O_5 and NH_3, and so on until at 56 the composition is 100 per cent K_2O. Likewise, plots on the line 1–56 receive no NH_3, series 3–57 receives 10 per cent NH_3, but varying amounts of P_2O_5 and K_2O, and so on until at 66 the composition is 100 per cent NH_3. Any circle within the triangle represents a mixture composed of the three constituents, its position in the triangle being determined by the composition, namely, the percentage of the three component parts, P_2O_5, NH_3, and K_2O. For instance, circle or plot 12 being on the 60 per cent phosphate line represents that composition of P_2O_5, namely 60 per cent, and being at the same time on the 10 per cent NH_3 line and 30 per cent K_2O line it represents 10 and 30 per cent of these constituents, respectively. The composition of the mixture represented by this circle is therefore P_2O_5 60 per cent, NH_3 10 per cent, and K_2O 30 per cent. The composition represented by each circle is given in table 1. The details of preparing the fertilizer mixtures according to the triangular system are fully explained in the publication cited.

The total amount of fertilizers applied annually was 50 pounds per acre of $P_2O_5 + NH_3 + K_2O$. The plot receiving acid phosphate only, received 50

pounds per acre of the constituent P_2O_5, the one receiving only potassium chloride received 50 pounds per acre of K_2O, and the one receiving sodium nitrate 50 pounds per acre of NH_3. The plots receiving two or three fertilizers, received the sum total of 50 pounds per acre of the constituents, $P_2O_5 + NH_3$, or $P_2O_5 + K_2O$, or $NH_3 + K_2O$, or $P_2O_5 + NH_3 + K_2O$, as the case may be. The fertilizers have been applied each year early in April soon after the grass began to grow.

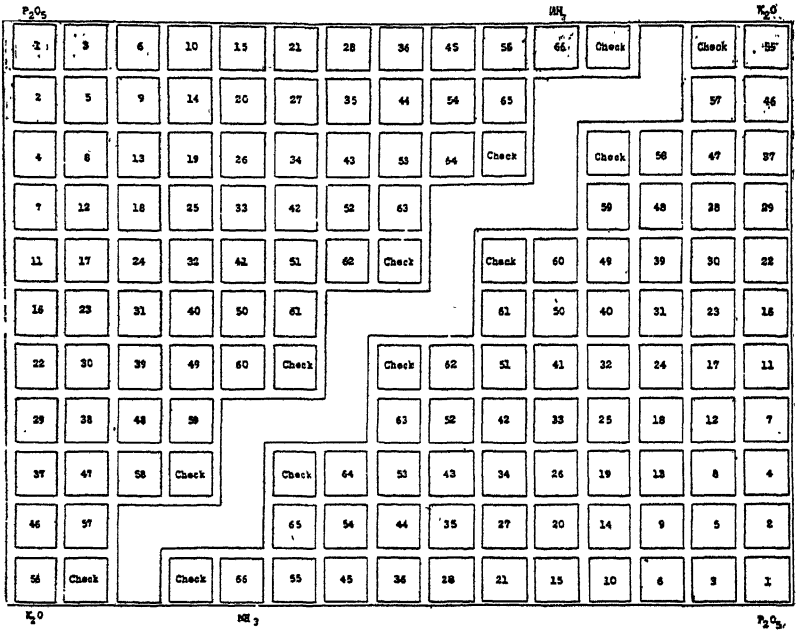
TABLE 1

Composition represented by each circle in plate 1

PLOT	FERTILIZER ADDED PER ACRE			PLOT	FERTILIZER ADDED PER ACRE			PLOT	FERTILIZER ADDED PER ACRE		
	P_2O_5	NH_3	K_2O		P_2O_5	NH_3	K_2O		P_2O_5	NH_3	K_2O
	pounds	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
1	50	0	0	23	20	5	25	45	10	40	0
2	45	0	5	24	20	10	20	46	5	0	45
3	45	5	0	25	20	15	15	47	5	5	40
4	40	0	10	26	20	20	10	48	5	10	35
5	40	5	5	27	20	25	5	49	5	15	30
6	40	10	0	28	20	30	0	50	5	20	25
7	35	0	15	29	15	0	35	51	5	25	20
8	35	5	10	30	15	5	30	52	5	30	15
9	35	10	5	31	15	10	25	53	5	35	10
10	35	15	0	32	15	15	20	54	5	40	5
11	30	0	20	33	15	20	15	55	5	45	0
12	30	5	15	34	15	25	10	56	0	0	50
13	30	10	10	35	15	30	5	57	0	5	45
14	30	15	5	36	15	35	0	58	0	10	40
15	30	20	0	37	10	0	40	59	0	15	35
16	25	0	25	38	10	5	35	60	0	20	30
17	25	5	20	39	10	10	30	61	0	25	25
18	25	10	15	40	10	15	25	62	0	30	20
19	25	15	10	41	10	20	20	63	0	35	15
20	25	20	5	42	10	25	15	64	0	40	10
21	25	25	0	43	10	30	10	65	0	45	5
22	20	0	30	44	10	35	5	66	0	50	0

The plots were laid off according to the triangular system as shown in figure 1. They were made 10 feet square and are separated by 2-foot paths. The plots were laid off in duplicate with a 10-foot area separating the two series, or triangles. Besides the 66 treated plots, in each series there are 6 check plots. From their relative positions, the two series in this discussion will always be referred to as the North Triangle and the South Triangle.

The hay was cut each year, early in July, and records made of the yield from each plot. The vegetation was allowed to grow, mature, and seed for the remainder of the season. No second cutting was made.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and is obtained by finding the radius corresponding to these numbers as areas of a circle, according to the formula: $A = \frac{R^2 \pi}{x}$ for which R can either be calculated or taken from a table of such values to be found in many books. The

TABLE 2

Average annual yield of cured hay per acre for seven years (1910–1916)

PLOT	FERTILIZER ADDED PER ACRE			NORTH TRIANGLE	SOUTH TRIANGLE	PLOT	FERTILIZER ADDED PER ACRE			NORTH TRIANGLE	SOUTH TRIANGLE
	P_2O_5	NH_3	K_2O				P_2O_5	NH_3	K_2O		
	pounds	*pounds*	*pounds*	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	50	0	0	1238	1207	34	15	25	10	1922	1569
2	45	0	5	1226	1152	35	15	30	5	1731	1637
3	45	5	0	1392	1702	36	15	35	0	1770	2377
4	40	0	10	1178	1646	37	10	0	40	1402	1413
5	40	5	5	1672	1613	38	10	5	35	1399	1699
6	40	10	0	1508	1502	39	10	10	30	1728	1612
7	35	0	15	1440	1101	40	10	15	25	1632	1563
8	35	5	10	1627	1561	41	10	20	20	2129	1554
9	35	10	5	1559	1411	42	10	25	15	1997	1482
10	35	15	0	1512	1200	43	10	30	10	2112	1806
11	30	0	20	1539	1233	44	10	35	5	1854	2424
12	30	5	15	1542	1609	45	10	40	0	1942	2602
13	30	10	10	1318	1369	46	5	0	45	1651	1344
14	30	15	5	1580	1507	47	5	5	40	1392	1516
15	30	20	0	1672	1249	48	5	10	35	1299	1560
16	25	0	25	1613	1250	49	5	15	30	1752	1672
17	25	5	20	1473	1498	50	5	20	25	2007	1453
18	25	10	15	1630	1488	51	5	25	20	2140	1536
19	25	15	10	1677	1084	52	5	30	15	2009	1660
20	25	20	5	1940	1479	53	5	35	10	1347	2198
21	25	25	0	1796	1723	54	5	40	5	1854	2373
22	20	0	30	1730	1289	55	5	45	0	1827	1926
23	20	5	25	1733	1421	56	0	0	50	1297	1233
24	20	10	20	1691	1346	57	0	5	45	1488	1420
25	20	15	15	1728	1151	58	0	10	40	1657	1360
26	20	20	10	1996	964	59	0	15	35	1710	1752
27	20	25	5	1957	1804	60	0	20	30	1610	1685
28	20	30	0	1912	1879	61	0	25	25	1834	1440
29	15	0	35	1643	1464	62	0	30	20	1968	1440
30	15	5	30	1407	1236	63	0	35	15	2120	1656
31	15	10	25	1710	1444	64	0	40	10	1910	1782
32	15	15	20	1795	1367	65	0	45	5	1903	2400
33	15	20	15	1651	1138	66	0	50	0	1728	2445

position of the circle indicates the fertilizer composition of the plot as explained in connection with plate 1.

Considering first the North Triangle, the relative yields of which are shown in figure 3, it is seen that the larger circles all occur in the nitrogen end of

the triangle, that is, in that region of the triangle where the fertilizer mixtures contain from 25 to 35 pounds per acre of NH_3, from 5 to 20 pounds of P_2O_5, and from 5 to 15 pounds of K_2O, namely the group of plots marked, 27, 34, 35, 42, 43, 44, 51, 52, 53, 54 (pl. 1). It is very noticeable that the circles which lie further from this group, either in the direction of the phosphate or potash end of the triangle become smaller.

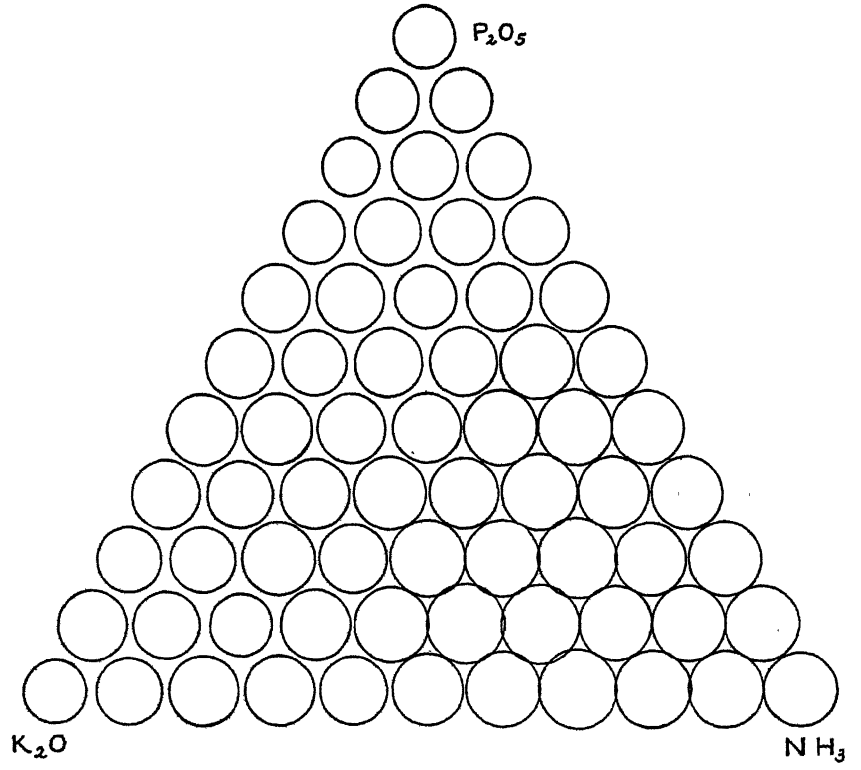

FIG. 2. DIAGRAM SHOWING THE RELATIVE AVERAGE YIELD OF VEGETATION OF THE PLOTS OF THE NORTH TRIANGLE FOR 7 YEARS, 1910–1916

The amount of hay from each plot is in proportion to the area of the circle. It is seen that the largest circles all occur in the nitrogen end of the triangle. Notice that the circles which lie further from the nitrogen end, either in the direction of the phosphate or potash end, become smaller.

The appearance of the circles representing the yields of the plots in the South Triangle, shown in figure 3, is similar in a general way to those representing the North Triangle. Here it is seen that the largest circles are in the extreme nitrogen end of the triangle, and like the circles of figure 2 grow smaller towards the phosphate or potash end. The largest yields in this experiment are in the plots which receive mixtures containing from 35 to 50 pounds per

acre of NH_3, from 5 to 10 pounds of P_2O_5, and from 0 to 10 pounds of K_2O. This group comprises plots 36, 44, 45, 53, 54, 55, 65, and 66.

Thus the two experiments agree in showing that the largest yields are produced by the fertilizer mixtures containing principally nitrogen, with relatively smaller amounts of phosphate and potash.

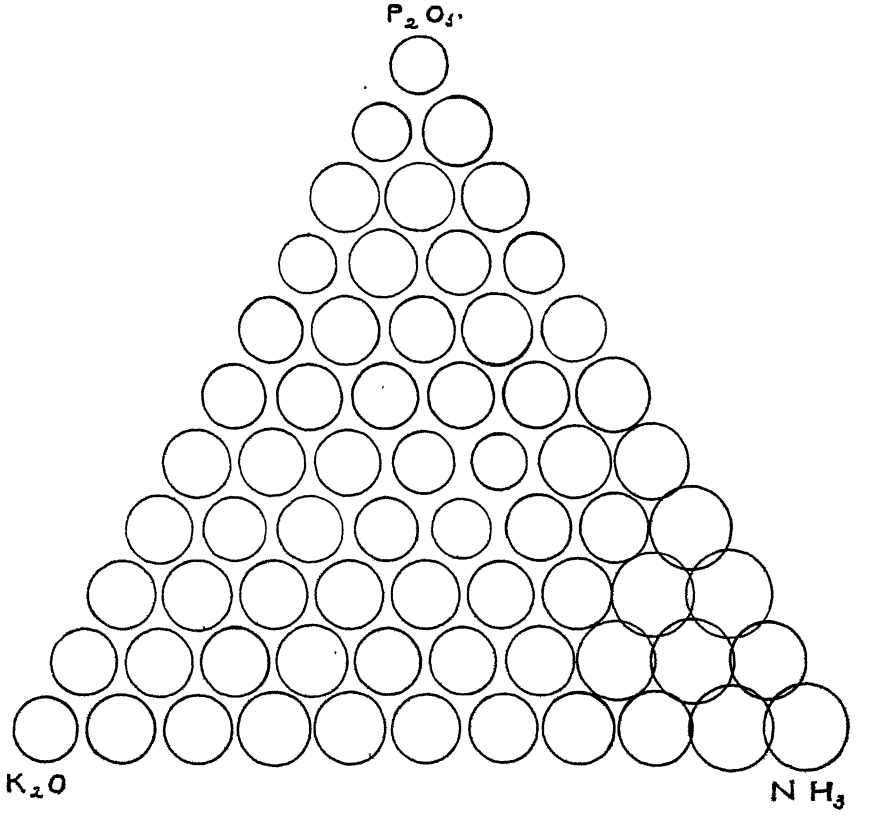

FIG. 3. DIAGRAM SHOWING THE RELATIVE AVERAGE YIELD OF VEGETATION OF THE PLOTS OF THE SOUTH TRIANGLE FOR 7 YEARS, 1910–1916

The amount of hay from each plot is in proportion to the area of the circle. Like the yield in its companion experiment (fig. 2) the largest circles all occur in the nitrogen end of the triangle, becoming smaller as one looks toward either the phosphate or potash section of the diagram.

INFLUENCE OF FERTILIZERS ON THE BOTANICAL COMPOSITION OF THE VEGETATION

The species of grasses which predominate in the differently fertilized plots present some interesting features. It should be recalled that the field was seeded to grass with wheat in 1906, and to clover in the spring of 1907. It

was pastured from 1908 to 1909, when the field was divided into plots and the annual application of fertilizers begun in 1910. At this date the entire area on which the plots are located had a fairly uniform stand of grass, consisting principally of Canada bluegrass (*Poa compressa*), Kentucky bluegrass (*Poa pratensis*), and timothy (*Phleum pratense*), with smaller amounts of white (*Trifolium repens*) and red clover (*Trifolium pratense*). The three grasses prevailed over the entire area in the proportion of about 50, 30, and 20 per cent, respectively. When the individual plots were studied in 1916 it was found that they were quite variable in the species which predominated, there being a relation between the composition of the vegetation and the fertilizer.

The dominance of each species in 1916, is shown graphically in figures 1 and 2, plate 2. The amount of each species had been determined for several years by going upon the plot before the hay was ready to cut and noting carefully the relative amounts of each kind of grass. In 1916 the record included also the percentage of white and red clover. The per cent of each species as found in 1916 is charted in the circles, representing each of the 66 plots. The three shades of green represent the three grasses, timothy, Kentucky bluegrass, and Canada bluegrass, and two shades of red the two clovers, white and red, as shown in the key on the charts.

North Triangle

Grasses. Considering first the composition of the vegetation on the North Triangle as shown in figure 1, it is seen that the Kentucky bluegrass is by far the most dominant of the grasses. This is especially true in the nitrogen end of the triangle. Here the other grasses have almost disappeared. In plot 66 which received only nitrogen fertilizers the grass is all of this species; and in plots 55 and 56, which receive 45 pounds per acre of NH_3, the grass is 95 per cent Kentucky bluegrass. This is also true in the next nitrogen line of plots, that is plots 45, 54 and 64. These plots receive a fertilizer mixture, containing 40 pounds of NH_3 per acre, with 10 pounds of the other constituents alone and together. The next line of nitrogen plots, 36, 44, 53 and 63, which receive a fertilizer mixture, containing 35 pounds per acre of NH_3, and 15 pounds of the other constituents, has in each case 90 per cent Kentucky bluegrass. Proceeding from the nitrogen end of the triangle the grasses, especially Kentucky bluegrass, diminish, giving way to clover. Canada bluegrass has disappeared from all of the plots, except 56 and 57, two plots which receive high potash fertilizers and are low in nitrogen and phosphorus. Small amounts of timothy have persisted in certain of the plots; it is perceptible that only a few plots in the nitrogen end of the triangle contain timothy, its occurrence being more frequent in the plots near the interior and towards the phosphate and potash ends of the triangle.

In 1912, the first year in which detail notes were made, the 21 plots in the nitrogen end of the triangle, that is those plots within the sub-triangle (pl. 1)

bounded by 66, 21 and 61 contained the three grasses, timothy, Kentucky bluegrass and Canada bluegrass in the proportion of 17, 52, and 31 per cent, respectively. In 1916 these 21 plots as a unit contained no Canada bluegrass, about 5 per cent of timothy, and approximately 77 per cent of the entire vegetation was Kentucky bluegrass. The average composition of the vegetation of these 21 nitrogen plots is shown in the small separate circle of figure 1, plate 2, marked 69.

In 1912 the relative amounts of the three grasses in the 21 mainly phosphate plots, those in sub-triangle, marked by plots 1, 16 and 21 was 14 per cent timothy, 25 per cent Kentucky bluegrass, and 61 per cent Canada bluegrass. As shown in circle 67, which gives the average composition of these 21 plots the Canada bluegrass has disappeared from this area; there is about 5 per cent of timothy and the amount of entire vegetation is 57 per cent of Kentucky bluegrass. White clover has gained considerable area in this section of field.

In 1912 the 21 plots receiving the high potash fertilizers, that is the area marked by plots 56, 16 and 61, contained the three grasses, timothy, Kentucky bluegrass, and Canada bluegrass in the proportion of 28, 28 and 44 per cent, respectively. An examination of figure 1, circle 68, giving the average composition of the grasses of these 21 plots in 1916, shows that Canada bluegrass has practically disappeared. Examination of the 21 plots in the diagram shows that it has disappeared in all but two of these plots. About 10 per cent of the entire area is timothy, and 44 per cent Kentucky bluegrass White clover has increased in this area.

From a study of the records of this experiment it would seem that in fertilizing a pasture containing mixed vegetation such as prevailed in this field, nitrogen is especially favorable to growth of Kentucky bluegrass, aiding it in its struggle for existence against its competitors. The relative amount of this variety is greater in the high nitrogen than in the high phosphate or high potash plots. This is seen by a general examination of the chart of figure 5 and also by circle 69, which shows the average composition of the 21 mainly nitrogenous plots after 7 years of fertilization. Timothy has prevailed in only a few of the plots in the nitrogen end of the triangle. In this experiment timothy has stood the struggle for existence best where fertilizers high in potash were used, this is shown in circle 68, which gives the average composition of the 21 mainly potassic plots. Canada bluegrass which was the prevailing grass when the experiment was started has disappeared in all but two plots, which shows that the conditions of this field resulting from fertilization or management are not suitable for this variety.

Clover. As seen in plate 2, fig. 1, only a small amount of clover prevails in the nitrogen end of the triangle, white clover is found more abundantly than red clover, in fact red clover has become extinct in all but one of the mainly nitrogen plots. Clover becomes more abundant as one proceeds from the nitrogen end of the triangle to the section of the field which receives

decreasing amounts of nitrogen, either in the direction of the phosphate or potash end.

Looked at from the point of view of the nitrogen content of the fertilizer, clover is more abundant in the line of plots 1–56, which receives mixtures of phosphate and potash, but no nitrogen, than any other set of plots. The next line of plots 3–57 which receives fertilizers containing in the mixture only 5 pounds of NH_3 are next in order from the point of view of the prevalence of clover. The dominance of clover becomes still less in the third line of plots 6–58, which receives fertilizer mixtures containing 10 pounds of NH_3, and likewise as one approaches those plots in the order of the increasing amount of nitrogen in the fertilizer mixture, the amount of clover is observed to decrease.

The extinction of red clover in the high-nitrogen plots and its prevalence in those plots fertilized with a mixture containing none or only a relatively small amount of nitrogen seems significant. It will be noticed that red clover is still found in almost all of the plots receiving a fertilizer mixture low in nitrogen.

Looked at from the point of view of the mainly nitrogenous, mainly phosphatic and mainly potassic plots it is seen that in the 21 mainly nitrogen plots, red clover has disappeared in all but one plot, but white clover still prevails in all but three. The average composition of the vegetation of these 21 plots has 16 per cent of white clover. The average composition of the vegetation of the 21 mainly phosphatic plots is 32 per cent white clover and 5 per cent red clover. In the 21 mainly potassic plots there is 32 per cent of white clover and 12 per cent of red clover.

SOUTH TRIANGLE

Grasses. The South Triangle presents a picture somewhat similar to the North Triangle when the percentages of the different species of grasses and clover, are charted in colors, as shown in plate 2, figure 2. Here again it is apparent that the grasses, and especially Kentucky bluegrass, predominate in the plots in the nitrogen end of the triangle. Proceeding from the nitrogen end of the triangle, the clover is more abundant.

In 1912 the 21 mainly nitrogen plots, as a whole, contained the grasses, timothy, Kentucky bluegrass and Canada bluegrass in the proportion of 5, 30 and 65 per cent, respectively. The average composition of the grass of these 21 plots as noted in 1916 is shown in circle 69 of plate 2, figure 2, where it is seen that 10 per cent of the entire vegetation is timothy, 60 per cent Kentucky bluegrass, and only 5 per cent Canada bluegrass. In these plots receiving fertilizers of high nitrogen content, timothy has increased slightly, Kentucky bluegrass has gained considerably, and Canada bluegrass has become almost extinct.

In 1912, timothy, Kentucky bluegrass, and Canada bluegrass prevailed on the 21 mainly phosphatic plots, in the proportion of 5, 20 and 75 per cent,

respectively. The average composition of the vegetation of these plots in 1916, as shown in the circle 67, plate 2, figure 2, is 5 per cent timothy, 40 per cent Kentucky bluegrass and 10 per cent Canada bluegrass. In these plots both timothy and Canada bluegrass still prevail in appreciable amounts. There is slightly more timothy, a large increase in Kentucky bluegrass, and a great decrease in Canada bluegrass.

The three grasses, in the 21 mainly potassic fertilizer plots in 1912, were timothy 17, Kentucky bluegrass 33 and Canada bluegrass 50 per cent. In 1916 the timothy has increased, 20 per cent of the entire vegetation being of this species, 40 per cent being Kentucky bluegrass, and 5 per cent Canada bluegrass.

Again in this experiment it is apparent that Kentucky bluegrass has become the predominating species. It is also apparent, as in its companion experiment, that the fertilizer mixtures high in nitrogen are especially favorable for the dominance of this species over other grasses and clover. This is seen by an examination of the circles within the sub-triangle 21–61–66 comparing them with the other circles of the triangle. It is also seen by an examination of circles 67, 68 and 69. Circle 69 gives the average composition of the vegetation of the 21 mainly nitrogenous plots.

Timothy is found in most of the plots, having been crowded out in only a few. It occurs in larger proportions in the plots receiving a fertilizer high in potash, which like the first experiment discussed, seems to show that this species can exist best in a pasture of mixed vegetation under such fertilization The timothy has increased somewhat in the potash section of the triangle.

Canada bluegrass is losing ground rapidly; it still occurs in a number of plots in different parts of the triangle, occurring less frequently in the potash section.

Clover. The prevalence of clover in the variously fertilized plots in 1916 in this triangle is very much the same as in the experiment already discussed. In the plots in the nitrogen end of the triangle there is a relatively small amount of clover, especially red clover. Both the red and white clover increase in the plots as the nitrogen decreases. White clover is most prevalent on the no potash plots and on the plots in the interior of the triangle, where the fertilizers applied contained nearly equal proportions of the three constituents. The red clover is most abundant again in the line of plots receiving no nitrogen and in the next line of plots which receives only 5 pounds of NH_3 in the fertilizer applied. In the circles 67, 68 and 69 in plate 2, figure 2, it is again seen that there is more clover in the phosphate and potash than in the nitrogen plots. The two experiments agree in showing that the growth of clover in mixed herbage has less chance for existence under conditions of high nitrogen fertilization, this being especially true of red clover. Red clover, in fact, has dominated most in both experiments where the fertilizer mixture used contained phosphate and potash, and no nitrogen, or in a mixture of the 3 constituents containing only a small proportion of nitrogen.

THE INFLUENCE OF FERTILIZER ON THE REACTION OF THE SOIL

The lime requirement of the soil from each individual plot was made, on samples taken in the fall of 1917. The reaction of the differently fertilized plots varies greatly, and some interesting results were secured. The determinations were made by the Veitch method (4) on a sample, secured by taking 5 borings from each plot with a soil auger. The data are given in figure 4, in pounds of CaO per acre required to neutralize the first 7 inches weighing 2,000,000 pounds.

The most striking feature about these results is that the plots which received mixtures of acid phosphate and potassium chloride, but no sodium nitrate have the greatest lime requirement. This is seen by observing the figures of the no nitrogen plots, that is the line of plots 1–56 (pl. 1). The next line of plots, 3–57, which receives a mixture of the three fertilizers containing only 5 pounds of NH_3 as sodium nitrate have a lime requirement less than those where no sodium nitrate is contained in the fertilizer mixture, but greater than the line of plots which receives 10 pounds of NH_3 as sodium nitrate in the mixture. In the section of the triangle which receives small amounts of sodium nitrate, and in the center, where the fertilizer mixture is well balanced, that is, contains about equal proportions of the 3 constituents, the soil is either neutral or has a very low lime requirement. The result with the sodium nitrate is probably due to the utilization of nitrate by the growing crop, leaving the base, sodium, an accumulation of which would tend to cause an alkaline condition in the soil. It was shown by one of us that sodium nitrate continually applied to the Susquehanna silt loam on the Arlington Experimental Farm, prevented the soil from becoming as acid as untreated soil, in fact, it is commonly accepted that soils fertilized year after year with sodium nitrate, become alkaline and assume a bad physical structure (3).

The neutral character of the plots fertilized with sodium nitrate, or a well-balanced fertilizer of nitrate, phosphate and potash , may possibly be due to the biological changes in the soil produced by such fertilization.

DISCUSSION AND SUMMARY

The results of a fertilizer experiment on grass on the Hagerstown loam soil, which has been conducted for 7 years are reported. The fertilizers used are acid phosphate, sodium nitrate, and potassium chloride. Each fertilizer was used alone, and in combination of twos and threes, the ingredients varying in 10 per cent stages. In all there were 66 treatments, as outlined in the triangle scheme of fertilizer experimentation. The total amount applied on each plot annually was 50 pounds per acre of the fertilizer elements P_2O_5, NH_3, or K_2O.

The plots are located in a pasture field where the composition of vegetation was originally Canada bluegrass, Kentucky bluegrass, timothy, and white and red clover. At the end of 7 years the differently fertilized plots contained

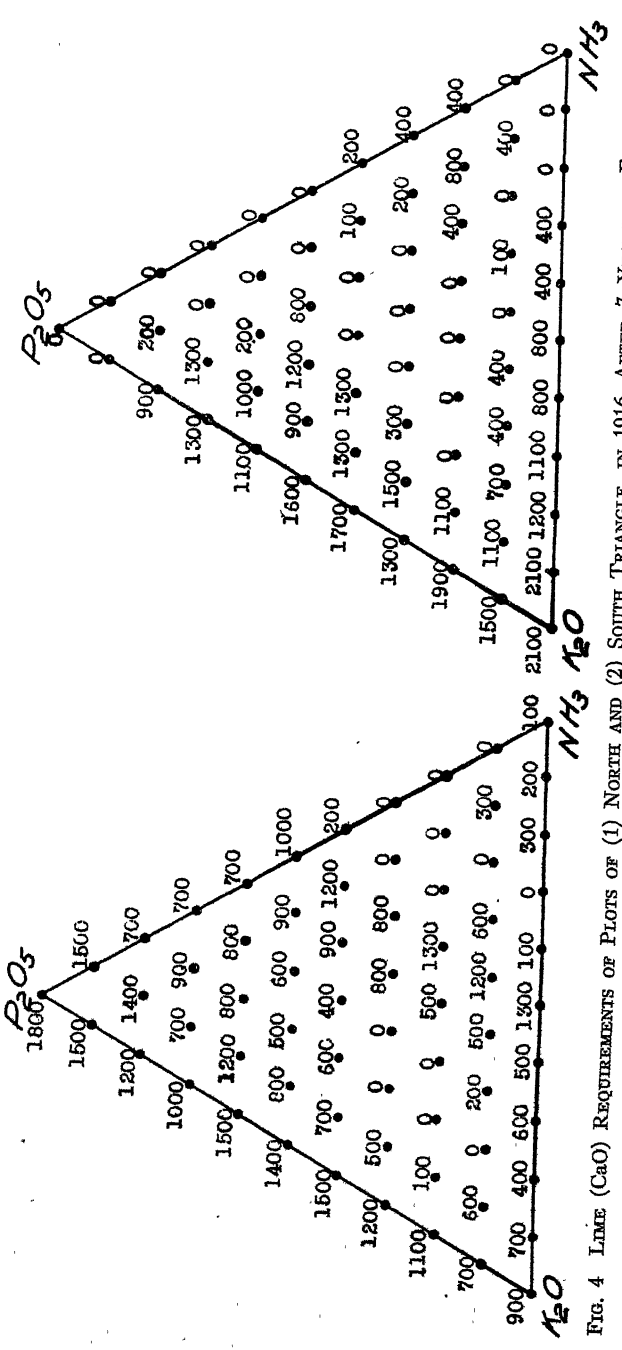

FIG. 4 LIME (CaO) REQUIREMENTS OF PLOTS OF (1) NORTH AND (2) SOUTH TRIANGLE, IN 1916, AFTER 7 YEARS OF FERTILIZATION, EXPRESSED IN POUNDS OF CaO REQUIRED TO NEUTRALIZE AN ACRE 6 INCHES

Note that the lime requirement of the high nitrogen plots is small, or neutral, whereas the plots receiving fertilizer mixture of low nitrogen ratios are higher, the lime requirement increasing as the sodium nitrate in the mixture decreases.

the various species in distinctly different proportion. Kentucky bluegrass has become the predominant variety, while in the beginning Canada bluegrass prevailed in larger proportion. Complete fertilizer mixtures high in nitrogen seem especially favorable for the dominance of Kentucky bluegrass over its competitors, while it is indicated that timothy is somewhat more favored by the fertilizers high in potash.

Grass generally has predominated over clover in the plots receiving fertilizers with high ratios of nitrogen, while clover and especially red clover has been crowded out of such fertilized plots. Clover has existed in the struggle for existence most easily in the plots fertilized with mixtures of phosphate and potash with no, or only a small amount of, nitrogen. The largest amount of clover occurs in the no nitrogen series of plots, and decreases in the plots as the nitrogen content of the fertilizer increases. This is very marked in the case of red clover.

The soil of the plots receiving well-balanced mixtures of acid phosphate, sodium nitrate and potassium chloride, or mixtures containing principally sodium nitrate tends to remain neutral or become alkaline, but where no sodium nitrate or only a small proportion is in the mixture the soil became acid. The lime requirement increases as the proportion of sodium nitrate in the fertilizer mixture used decreases.

It might be interpreted from these results that clover has stood the struggle for existence better in the plots which have become acid. However, this condition probably is not due to the reaction of the soil, but is due in part to the supremacy of Kentucky bluegrass in this section of the triangle where the fertilizers supplied the needed nitrogen for the grass and it succeeded in crowding out the clover. The conditions in the no nitrogen section of the triangle, were unsuited to grass, the clover predominating as it supplied its own nitrogen supply through root nodules.

The most acid plots are not sufficiently acid to prevent the growth of clover in this soil. White (5) has shown that red clover grows best in this soil when limed, the maximum yield was secured when limestone was applied at the rate of 3 tones per acre in excess of neutrality. Clover failed in field and in pot experiments where the lime requirement reached 3,000 to 3,500 pounds per acre. From the data in figure 4 it is seen that the lime requirement of the most acid plots is far less than these amounts found by White in his work on the Hagerstown loam. The predominance of species found to exist after 7 years of fertilizer application, is more probably due to the survival of that species for which the existing fertilizer treatment is best suited.

At the beginning of the experiment the dominant species was Canada bluegrass, while at the end of 7 years this grass generally has given way to Kentucky bluegrass, this variety now being the dominant grass, which may possibly be due to a superior power of endurance in the plant itself compared with its competitor, rather than to directly favorable conditions of fertilization. Other species are dominant on some plots, entirely absent in others,

or exist only in small proportion in still others. In such cases it may be assumed that the particular fertilizer has been either directly favorable to the species, or indirectly so by being prejudicial to the growth of others. If any one species be dominant on a group of plots more or less similarly fertilized, the inference is that the fertilizer is directly beneficial to that species.

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PLATE 1

THE FERTILIZER RATIOS USED IN THE GRASS EXPERIMENT, ARE SHOWN HERE BY THE TRIANGULAR DIAGRAM

There are 66 fertilizer mixtures, the constituents, acid phosphate, sodium nitrate and potassium chloride are used singly, in combination of twos, and in combinations of threes, the ratios of P_2O_5, NH_3, and K_2O vary in 10 per cent stages. Fertilizers were applied annually in amounts of 50 pounds per acre of P_2O_5, NH_3 or K_2O.

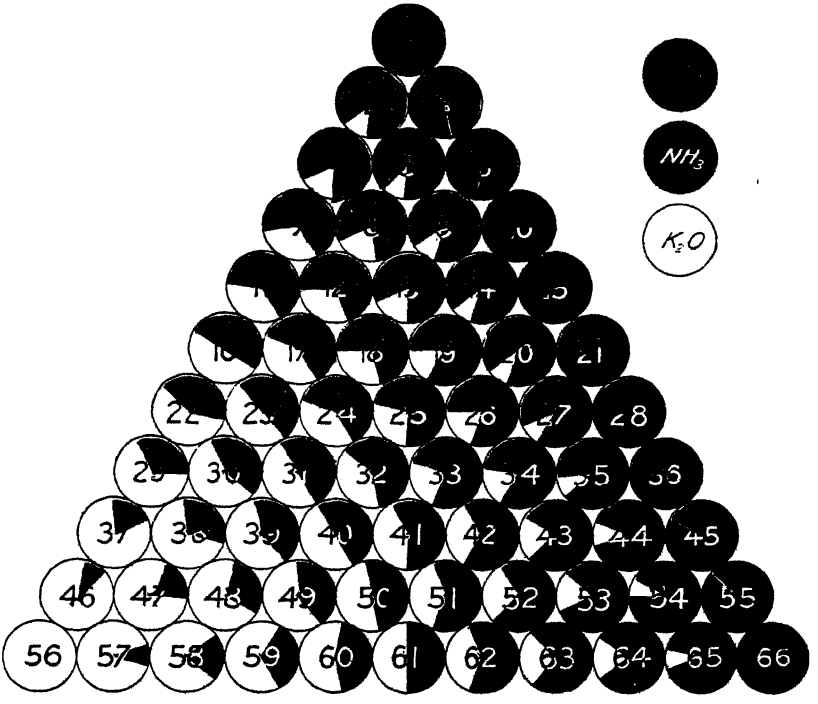
NH3
K2O

PLATE 2

FIG. 1. DIAGRAM SHOWING THE EFFECT OF FERTILIZERS, CONTAINING THE CONSTITUENTS, ACID PHOSPHATE, SODIUM NITRATE AND POTASSIUM CHLORIDE IN DIFFERENT RATIOS ON THE BOTANICAL COMPOSITION OF THE VEGETATION OF A PERMANENT PASTURE, AFTER 7 YEARS OF FERTILIZER TREATMENTS, IN THE NORTH TRIANGLE

Note that Kentucky bluegrass predominates in the nitrogen end of the triangle, and that this section contains almost no clover. Clover and especially red clover is more prevalent in the no nitrogen section of the field. Circle 67 shows the average composition of the 21 mainly phosphatic plots (sub-triangle 1–16–21), no. 68 the average composition of the 21 mainly potassic plots (sub-triangle 16–61–56), and no. 69 the average composition of the 21 mainly nitrogenous plots (sub-triangle 21–61–66).

FIG. 2. DIAGRAM SHOWING THE EFFECT OF FERTILIZERS, CONTAINING THE CONSTITUENTS, ACID PHOSPHATE, SODIUM NITRATE, AND POTASSIUM CHLORIDE IN DIFFERENT RATIOS ON THE BOTANICAL COMPOSITION OF THE VEGETATION OF A PERMANENT PASTURE AFTER 7 YEARS OF FERTILIZER TREATMENT, IN THE SOUTH TRIANGLE

It is also seen here that grass is the prevailing species in the plots receiving fertilizers with high nitrogen ratics, while clover has become almost extinct. Clover is again found more abundantly in the no nitrogen plots, and in the plots receiving fertilizer mixtures of a low nitrogen ratio. Circle 67 shows the average composition of the 21 mainly phosphatic plots (sub-triangle 1–16–21), no. 68 the average composition of the 21 mainly potassic plots (sub-triangle 16–61–56), and no. 69 the average composition of the 21 mainly nitrogenous plots (sub-triangle 21–61–66).

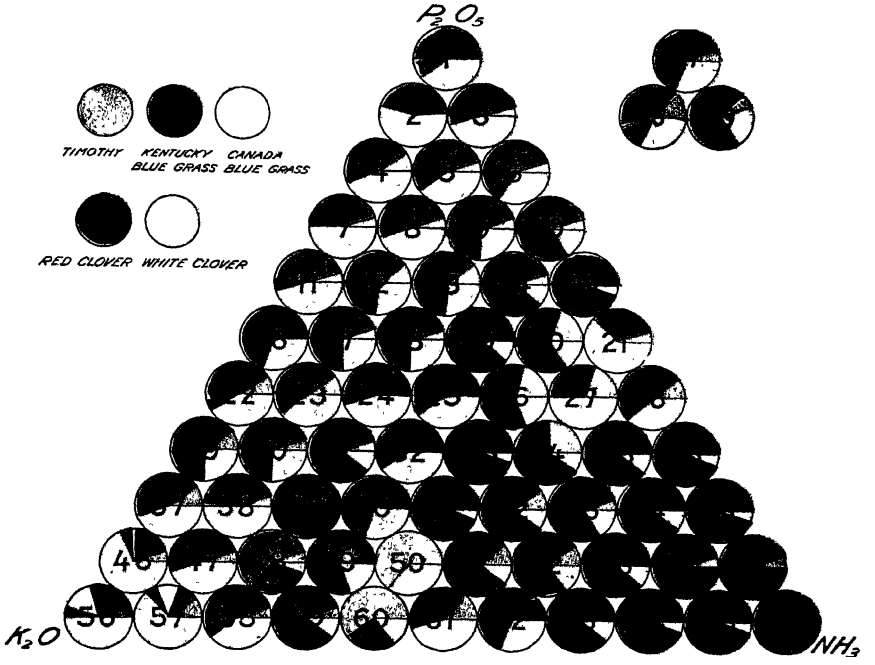
P_2O_5
TIMOTHY
KENTUCKY BLUE GRASS
CANADA BLUE GRASS
RED CLOVER
WHITE CLOVER
K_2O
NH_3

FIG. 1

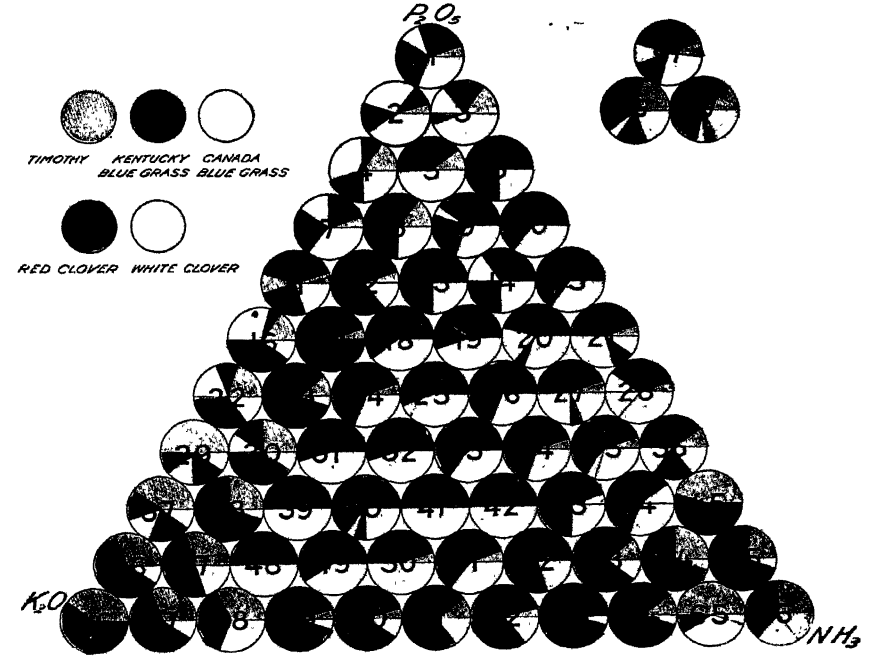
P_2O_5
TIMOTHY
KENTUCKY BLUE GRASS
CANADA BLUE GRASS
RED CLOVER
WHITE CLOVER
K_2O
NH_3

FIG. 2

ADJUSTING THE SOIL REACTION TO THE CROP

(*Editorial*)

Potato growers in New Jersey prefer acid soils for their crop. They justify this attitude by the claim that pronounced acidity in the soil assures to them a relative freedom from potato scab. That their claim is essentially sound is borne out not alone by the accumulated experience of many individuals, but also by adequate and convincing experimental study.

The potato growers recognize, at the same time, that a high lime requirement is entirely contrary to the effective and economical maintenance of the soil nitrogen supply. Whenever the lime requirement passes a certain point for any given soil type the growing of clover and alfalfa become increasingly difficult, and the accumulation in the soil of atmospheric nitrogen is accordingly depressed. Hence it becomes necessary to reconcile two opposing tendencies in the growing of large crops of clean tubers. It becomes desirable to create a pronounced acid reaction for the potato crop itself and later to reduce the acidity to a degree that would allow the successful growing of the lime-loving legumes.

A means to this end may be found in powdered sulfur. It would be quite feasible to apply in the early spring, or late in the preceding fall, a sufficient quantity of sulfur to establish any desired degree of acidity for the potato crop. Quantities of from 300 to 1000 pounds per acre of powdered sulfur could thus be employed, the exact amount to be determined by the physical and chemical make-up of each soil. After the potato crop is harvested the existing acidity may be ascertained and corrected by the use of lime. In this manner the soil reaction of any field may be varied from year to year to meet the needs of the potato crop, as well as of the legumes. In a more general way the same method may be employed to maintain such soil-reaction as may seem best, after proper experimental study, for any crop on any soil type.

JACOB G. LIPMAN.

EFFECT OF SULFOFICATION AND NITRIFICATION ON POTASSIUM AND OTHER SOIL CONSTITUENTS

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The experimental work reported in this paper is in part a continuation of investigations pertaining to the effect of sulfur on soils and crops, started by the writers in 1912. In a previous publication (1) we stated that the beneficial effects following the addition of sulfur to soils may result from an increased solubility of soil constituents due to acidity developed from oxidation of sulfur. Some experimental data were also reported at that time, showing that the addition of sulfur to a mixture of rock phosphate and soil increased the acidity of the water extract and the solubility of the phosphate. More recently considerable attention has been given to the influence of sulfofication and nitrification on the availability of rock phosphate.

Microorganisms are considered to be important agencies affecting chemical transformation in soils, but information as to the degree to which the solubilities of soil minerals are changed by organisms is rather limited. The results of different investigations have furnished indications that acids, other than carbonic acid, which are the products of bacterial activities have an appreciable solvent action on calcium. The products of the processes in which various groups of sulfur bacteria, and those producing changes in nitrogen compounds have a part, no doubt react with, and bring about changes in the solubility of other constituents, which may ultimately have an important rôle in crop production.

PROCEDURE

Soil mixtures which included the treatments given in the tables of results were maintained at a temperature of 30°C. for 19 weeks. After the various materials were incorporated with 500-gm. portions of soil, water was added to satisfy 60 per cent of the water-holding capacity, and the mixtures transferred to quart jars which served as containers. Duplicate mixtures of all the treatments were prepared. After the addition of water at regular intervals the contents of the jars were thoroughly mixed.

For the determination of water-soluble constituents, solutions were obtained by extracting 400-gm. portions with 2500 cc. distilled water for 15 hours and filtered through Berkefeld filters under pressure. The soils used in

the work were a silt loam deficient in bases, and a black clay soil. While the calcium carbonate content of the clay is only 300 parts per million, the soil is decidedly basic owing to the presence of basic calcium compounds other than carbonate.

The data reported include determination of potassium, calcium, magnesium, manganese, and aluminum. Indications of the extent to which sulfofication has proceeded are furnished by the quantities of water-soluble sulfur as sulfates. Changes which occurred in the nitrogen content are shown by the amounts of nitric and ammoniacal nitrogen which had accumulated in the soil mixtures at the expiration of the experimental period. Potassium and manganese were determined colorimetrically. Nitric nitrogen was determined by reduction with Devardas alloy and distillation. The usual gravimetric procedures were employed for calcium, magnesium, aluminum and water-soluble sulfur.

Tests were made for iron, but none was present in the solutions excepting a slight trace in two of the mixtures where the acidity was the greatest.

The amounts of the acid and basic ions in the water solutions of the soil mixtures, do not furnish complete evidence of the extent to which reactions may have occurred during the period intervening between the addition of the treatment, and the time the soils were extracted. However, the decided variations found are sufficiently indicative of the effect of the processes on soil constituents. The data for sulfur, acidity, nitrates and ammoniacal nitrogen are given in tables 1 and 2.

ACIDITY OF SOLUTION

A measure of the relative acidity of the solutions was obtained by titration using methyl red as the indicator. The data in tables 1 and 2 show that none of the solutions were acid to methyl red except those from the soils to which sulfur was added, and in these cases it was decidedly decreased where calcium carbonate was present, being practically all neutralized in the case of the silt loam when calcium carbonate was added at the rate of 8000 parts per million of soil.

The amounts of water-soluble ammoniacal nitrogen found where dried blood was present also furnish evidence that the ammonia formed has neutralized a part of the acidity developed by oxidation of sulfur.

All of the solutions of the mixtures from which sulfur was omitted had an alkaline reaction, the alkalinity being somewhat increased by addition of calcium carbonate, and by calcium silicate and blast furnace slag, these three materials supplying approximately equal amounts of calcium. Although the silt loam soil had a lime requirement equivalent to 4000 parts per million of calcium carbonate, the titration of the water extract gives no indication of a deficiency in bases necessary for the maintenance of an alkaline reaction in this soil. Ammonium sulfate has slightly decreased the alkalinity of the

TABLE 1

Silt loam soil

(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	SULFUR	NITRATE NITROGEN	AMMONIACAL NITROGEN	ACIDITY*	ALKALINITY*
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	37	84	13	0	3
2	Calcium carbonate 2 gm.	50	128	0	0	15
3	Dried blood 4 gm.	58	294	67	0	3
4	Dried blood 4 gm., calcium carbonate 2 gm.	95	462	0	0	14
5	Dried blood 4 gm., calcium silicate 2 gm.	122	434	6	0	8
6	Dried blood 4 gm., blast furnace slag 2 gm.	106	478	17	0	4
7	Ammonium sulfate 4 gm.	1,852	54	1,239	0	1
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	1,689	569	178	0	3
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	2,070	100	1,014	0	6
10	Sulfur 2 gm.	2,143	6	50	338	0
11	Sulfur 2 gm., calcium carbonate 4 gm.	1,294	35	27	4	0
12	Sulfur 2 gm., calcium carbonate 2 gm.	1,923	21	46	196	0
13	Sulfur 2 gm., dried blood 4 gm.	1,365	6	246	116	0
14	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	1,559	228	132	10	0
15	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	1,689	29	302	54	0
16	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	1,551	6	279	84	0
17	Sulfur 2 gm., dried blood 4 gm., feldspar 8 gm.	2,053	21	455	166	0
18	Sulfur 2 gm., dried blood 4 gm., feldspar 8 gm. calcium carbonate 4 gm.	1,256	373	172	0	0

*Acidity expressed as H_2SO_4, and *alkalinity as $CaCO_3$.

TABLE 2

Basic clay

(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 400 GM. DRY SOIL	SULFUR	NITRATE NITROGEN	AMMONIACAL NITROGEN	ACIDITY*	ALKALINITY*
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	87	153	3	0	8
2	Dried blood 4 gm.	90	491	25	0	3
3	Ammonium sulfate 4 gm.	1,696	280	737	0	1
4	Sulfur 2 gm.	2,156	16	67	42	0
5	Sulfur 2 gm., dried blood 4 gm.	2,225	12	279	17	0
6	Sulfur 2 gm., ammonium sulfate 4 gm.	3,480	42	1,071	30	0

*Acidity expressed as H_2SO_4, and *alkalinity as $CaCO_3$.

water solutions of both the silt loam soil and the basic clay. The reduction of alkalinity imparted to the solution of the silt loam by calcium carbonate was more pronounced.

The difference in the basicity of the two soils is clearly shown by a comparison of the figures for the acidities of the solutions from the sulfur-treated silt loam, and basic clay soil mixtures. The acidity of the silt loam soil and sulfur mixture is 338. Sulfur added to the basic clay has changed the reaction of the water solution to methyl red from an alkalinity of 8 parts per million to an acidity of 42 parts per million. Practically the same amounts of sulfates, 2143 and 2156 parts per million were found in the solutions from both the silt loam and the basic soil treated with sulfur. This quantity of sulfur is equivalent to 6560 parts per million sulfuric acid, which would require 6690 parts per million of calcium for neutralization. This requirement was largely provided for by calcium and magnesium in the basic clay. A total of 2055 parts per million, calcium and magnesium, was water-soluble in the basic clay, as compared with 473 for the silt loam soil. Calcium and magnesium being deficient in the silt loam, the acidity resulting from oxidation of sulfur has combined with considerable amounts of aluminum and manganese, the sulfates of these elements exceeding the water-soluble calcium and magnesium. Nitrification of dried blood has not caused the water solution of mixtures where nitrates were present to have an acid reaction to methyl red.

The figures obtained for the relative acidity of the solutions do not give an indication of the total acidity produced during the period that sulfur, dried blood, and ammonium sulfate were in contact with soil in the several mixtures. In considering the effects of acidity due to sulfofication or nitrification. it is obvious that the water-soluble sulfates and nitrates will furnish a more correct basis for any deductions made, for the reason that the products of these processes have combined with the soil constituents. The acidity has been neutralized chiefly by calcium carbonate where it was included as a part of the treatment in the silt loam mixtures, and by the natural basic calcium compounds of the black clay.

According to the figures for water-soluble sulfur, approximately 50 per cent of sulfur added at the rate of 4000 parts per million of soil was oxidized where no other additions were made. The effect of calcium carbonate when added in excess of the lime requirement of the silt loam soil, has been to depress the oxidation of sulfur. When half this amount of calcium carbonate was present, the oxidation of sulfur increased, but was less than occurred where no additions other than sulfur were made.

Some of the reactions which occurred between oxidation products of sulfur, and soil constituents in these experimental mixtures will no doubt proceed in field soils, although less actively, and are not dependent on the presence of added sulfur, as sulfur compounds in the soil can be a source of acidity under certain conditions.

Iron sulfide which is a mineral common to many soils is gradually oxidized to the form of sulfate, and then dissolved and hydrolized, with the precipitation of ferric hydrate and the formation of free sulfuric acid.

Sulfur existing in organic combinations may also be changed into a form which can react with soil bases, if the conclusions of Kappen and Quensell (5) concerning the sulfur cycle in soil are correct. They state that hydrogen sulfide produced by action of bacteria, combines with iron-forming iron sulfide which in turn is decomposed, the iron being oxidized and sulfur liberated in a finely divided condition which is oxidized, forming sulfates.

The data for nitric nitrogen show that the formation of nitrates has varied considerably depending on the treatment, nitrification having been stimulated by calcium carbonate, and depressed by the presence of sulfur. Addition of calcium carbonate to the soil deficient in basic calcium compounds has favored the nitrification of its natural supply of nitrogen, as well as that furnished by dried blood and ammonium sulfate.

By comparing the nitric nitrogen results for the three mixtures in which the same amounts of calcium carbonate, calcium silicate and blast furnace slag were included, it will be observed that these three materials have been equally satisfactory for supplying calcium necessary to combine with nitrous acid.

The production of nitric nitrogen from dried blood exceeded that from ammonium sulfate in the basic clay, and in the silt loam soil having no addition of calcium carbonate; the amount formed from ammonium sulfate was less than the accumulation in the untreated soil. The increased base requirement for nitrification of ammonium sulfate as compared with dried blood is evidenced by the nitrate results for both soils. Where calcium carbonate was added to the silt loam at the rate of 4000 parts per million of soil, the formation of nitric nitrogen from dried blood was 462 in contrast with 100 parts per million from ammonium sulfate. When calcium carbonate was supplied at the rate of 8000 parts per million, more nitrates were produced from ammonium sulfate than from dried blood. The natural supply of basic calcium in the clay soil supported a more vigorous nitrification of ammonium sulfate than occurred in the silt loam soil, but the quantity of nitric nitrogen found was less than that from dried blood in this soil.

Acidity resulting from oxidation of sulfur has depressed nitrification. When sulfur and dried blood were in contact, and no calcium carbonate supplied, nitrification was practically inhibited. Where conditions resulting from sulfofication and absence of sufficient base prevented the complete change from proteid to nitric nitrogen, the ammonia formed has partly neutralized acidity.

POTASSIUM SOLUBILITY

The results obtained for water-soluble potassium are of interest because they furnish indications of the effect of salts formed, as the result of acidity developed by micro-organisms, which produce changes in sulfur and nitrogen

carriers. This effect on potassium compounds in the soil, appears to have been a more important factor than the direct action of acidity resulting from the processes of either nitrification or sulfofication.

The solubility of potassium in soil and potassium-bearing minerals as affected by various salts, is a subject to which much attention has been directed. Sodium salts, including nitrate and chloride, liberate potassium according to some investigators, while the opposite conclusion has been arrived at by others. Many conflicting statements also are presented in the literature pertaining to the effect of lime, calcium carbonate, calcium sulfate, and other calcium salts on its solubility. Gardiner and Shorey (4) found that potassium of muscovite and orthoclase was rendered soluble to some extent by treatment with various concentrations of ammonium sulfate solution.

The work of Morse and Curry (9) indicate that lime and gypsum increased the solubility of potassium in feldspar. The experimental work of Bradley (2) with Oregon soils shows that with some soils there was an increase of water-soluble potassium brought about by the addition of gypsum. Investigations reported by McMiller (8) show that various mineral soils mixed with 1 per cent of gypsum, and the water content brought to a point approximating the moisture equivalent and kept in this condition for three months gave marked increases in water-soluble potassium.

Results obtained by Ruprecht and Morse (11) in an investigation of Massachusetts soils which had received additions of ammonium sulfate as a part of the fertilizer treatment, show that there has been a slight solvent action on potassium in the absence of lime.

Lipman and Gericke (6) from experiments conducted for the purpose of determining how calcium carbonate and calcium sulfate affect the solubility of soil constituents, conclude that all soils do not behave alike when treated with these materials. Their results showed an increased solubility in some soils but not in others. The conclusions of Briggs and Breazeale (3) based both on the results of the analyses of the solutions, and on the measurement of the potassium content of wheat seedlings grown in the solutions, are that the availability to plants of the potassium in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum. They found in some instances a marked depression of the solubility in the presence of gypsum.

The results obtained in our experimental work on the effect of nitrification and sulfofication on soil constituents, show that with certain conditions of treatment, there has been some effect on the potassium compounds in the soil, although the amounts of potassium obtained in water solutions are generally much less than the quantities of some of the other elements determined. Considered collectively, the data for potassium, calcium, and sulfur in solution, as well as the ammoniacal nitrogen produced under certain conditions of treatment, indicate that potassium has been liberated chiefly by calcium sulfate and ammonium sulfate.

The data for soluble potassium which are given in tables 3 and 4 show that the process of nitrification, considered from the viewpoint of nitrous acid as a solvent, has affected the solubility less than where the transformation of organic nitrogen occurred in the presence of oxidized sulfur. Whatever effect the change in the nitrogen furnished by the treatment with dried blood has had, may be due to calcium or other nitrates formed, rather than to the

TABLE 3

Effect of nitrification on potassium in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	NITRATE NITROGEN
		p.p.m.	*p.p.m.*
1	None	18	84
2	Calcium carbonate 2 gm.	7	128
3	Dried blood 4 gm.	30	294
4	Dried blood 4 gm., calcium carbonate 2 gm.	24	462
5	Dried blood 4 gm., calcium silicate 2 gm.	33	434
6	Dried blood 4 gm., blast furnace slag 2 gm.	39	478
7	Ammonium sulfate 4 gm.	30	54
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	21	569
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	26	100

TABLE 4

Effect of nitrification and sulfofication on potassium in basic clay soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	NITRATE NITROGEN	SULFUR
		p.p.m.	*p.p.m.*	*p.p.m.*
1	None	31	153	87
2	Dried blood 4 gm.	44	491	90
3	Ammonium sulfate 4 gm.	72	280	1,696
4	Sulfur 2 gm.	48	16	2,156
5	Sulfur 2 gm., dried blood 4 gm.	60	12	2,225
6	Sulfur 2 gm., ammonium sulfate 4 gm.	85	42	3,480

direct action of acidity. In the acid and basic soils used in this investigation, the addition of dried blood has caused a small increase of water-soluble potassium.

It will be noted from the results in table 5, showing the effect of sulfofication on potassium in the acid silt loam, that where dried blood and sulfur were the additions made, a further increased solvent action has taken place. This is considered to be the result of the action of ammonium sulfate, since in the presence of sulfur gradually being oxidized, the ammoniacal nitrogen formed has combined with the sulfur acidity. The quantity of ammoniacal nitrogen found at the end of the experimental period was 246 parts per million.

The amount actually present, however, was in excess of this, for ammonia added to soil cannot all be recovered except by distillation with magnesium oxide. The addition of sulfur and dried blood to the basic soil has affected the solubility of potassium in a similar manner. The potassium obtained in solution from the acid silt loam mixtures which included dried blood and sulfur are given in table 5, and the results for the mixtures to which either dried blood or ammonium sulfate were added are shown in table 3.

In the acid silt loam the addition of ammonium sulfate has not affected the solubility of potassium to the same extent as the transformations of sulfur and dried blood when added together, which are considered to have formed ammonium sulfate. The potassium solubility of the basic clay mixtures which are presented in table 4 show that added ammonium sulfate has had an appreciable effect; the increases in this soil being greater than those found in the water solution from the acid soil where ammonium sulfate was added.

TABLE 5

Effect of sulfofication on potassium in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	SULFUR	ACIDITY*
		p.p.m.	*p.p.m.*	*p.p.m.*
1	Sulfur 2 gm.	18	2,143	338
2	Sulfur 2 gm., calcium carbonate 4 gm.	32	1,294	4
3	Sulfur 2 gm., calcium carbonate 2 gm.	53	1,923	196
4	Sulfur 2 gm., dried blood 4 gm.	52	1,365	116
5	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	43	1,559	10
6	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	49	1,689	54
7	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	62	1,551	84

*Acidity expressed as H_2SO_4.

Considering the effect of oxidation of sulfur on potassium, the results show that oxidized sulfur has produced less solubility in the acid soil than in the basic clay. The effect of sulfur treatment alone, and when added with blood and calcium carbonate, is shown by the results for the acid soil in table 5 and for the basic clay in table 4. It will be noted that in the acid soil, there has been no increase of water-soluble potassium where sulfur was the only addition, but when included with calcium carbonate or dried blood the solubility of the potassium was found to be appreciably increased.

Sulfofication proceeded no less actively in the absence of calcium carbonate and dried blood, in fact the quantity of sulfur from the acid soil mixture treated with sulfur alone was greater than when these materials were added. The acidity of the water extract was also greater when dried blood and calcium carbonate were not added.

The formation of ammonium sulfate has been offered as the explanation of the effect of sulfur when used with dried blood. With calcium carbonate

added, the acidity from the oxidation of sulfur has reacted with calcium carbonate and a considerable amount of calcium sulfate has been formed in the mixtures. This is evident from the amounts of water-soluble calcium obtained from the silt loam mixtures receiving additions of calcium carbonate and sulfur, as well as in the basic clay where no calcium carbonate was supplied. The amounts of water-soluble calcium found in the various mixtures, including those treated with sulfur are presented with the data in tables 7 and 8.

The presence of calcium sulfate in the mixtures which have just been discussed is considered to have been the agency chiefly responsible for the liberation of potassium from the insoluble combinations in which it existed in these soils.

TABLE 6

Solubility of potassium supplied by feldspar in sand and soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. SAND AND SOIL	POTASSIUM	NITRATE NITROGEN	AMMONIACAL NITROGEN	SULFUR
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Sand	0	0	0	0
2	Sand, sulfur 2 gm.	0	0	0	42
3	Sand, sulfur 2 gm., calcium carbonate 2 gm.	0	0	0	503
4	Sand, feldspar 8 gm.	4	0	0	0
5	Sand, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm.	52	27	325	478
6	Sand, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm., calcium carbonate 2 gm.	33	21	58	1,937
7	Soil	18	84	13	37
8	Soil, dried blood 4 gm., sulfur 2 gm.	52	6	245	1,365
9	Soil, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm.	96	21	465	2,053
10	Soil, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm., calcium carbonate 2 gm.	58	373	172	1,256

The general conception of the action of calcium sulfate in liberating potassium, has been that this phenomenon is brought about through the exchange of calcium with other soil bases. A more recent hypothesis has been advanced by Nolte (10) who considers that calcium sulfate acts on the soil by means of both its constituents, double decomposition occurring with the mineral compounds of the soil.

Where the soils were treated with sulfur, dried blood and calcium carbonate in the case of the acid silt loam, there was no doubt a combined effect of ammonium sulfate and calcium sulfate. Calcium nitrate produced where nitrification of dried blood occurred, especially in the presence of calcium carbonate, calcium silicate and blast furnace slag, may have had an effect on potassium compounds in the soil.

Since soluble potassium salts added to soils are very rapidly fixed to such an extent that only small quantities can be recovered by extraction with water, it is very probable that the amounts of potassium obtained do not

represent the total quantities which may have been changed from insoluble compounds in the soil, to more soluble forms, by the processes taking place in the soil mixtures during the period of incubation.

Further indications of the effect produced by nitrogen and sulfur transformations are furnished by data obtained where feldspar was included with sand and with soil. The treatment and the amounts of water-soluble potassium obtained are stated in table 6. Dried blood and sulfur where 8 gm. of feldspar was added to 500-gm. portions of sand has decidedly increased the solubility of potassium of the feldspar. With calcium carbonate included in the treatment, solubility is decreased, and a similar effect has taken place where feldspar was included with dried blood and sulfur in an acid silt loam soil mixture.

SOLUBLE CALCIUM AND MAGNESIUM

It is apparent from the results for calcium in tables 7 and 8 that considerable amounts, present as combinations other than carbonate, have been changed to more soluble compounds in the differently treated soil mixtures. Where dried blood was nitrified in the silt loam soil, which is deficient in basic calcium, the calcium in solution was 255 parts per million as compared with 111 parts per million from the untreated soil. The addition of calcium carbonate stimulated nitrification, consequently a larger amount of calcium nitrate was formed. Calcium supplied as silicate, and by blast furnace slag was made soluble in a similar manner.

The calcium in solution from the basic clay soil was dissolved from its natural supply, since no calcium carbonate was added to this soil which contains a large amount of readily salifiable base, most of which is evidently combined as silicate, and a small proportion as carbonate. The total calcium content of this soil is 9400 parts per million as compared with 2300 in the other soil. Nitrification of dried blood, and the original nitrogen of the clay soil, proceeded to the extent that the soluble calcium was considerably in excess of the amounts extracted from similar mixtures of the silt loam. Lyon and Bizzell (7) have reported data which show that a large removal of nitrates from an unplanted soil, where conditions were favorable for nitrification, was associated with a correspondingly large loss of calcium.

Ammonium sulfate, independent of the ammonia being nitrified, reacted with calcium either originally present or added as carbonate, and the solutions of the soil mixtures in which this treatment was included contained large amounts of calcium. These results for soluble calcium, corroborate the information obtained from the analyses of drainage water from soils which have received application of ammonium sulfate, that this material depletes the supply of basic calcium compounds.

Acidity resulting from the oxidation of sulfur has freely attacked the soil constituents, including calcium, the amount in solution varying with the

TABLE 7

Data for calcium, magnesium, aluminum and manganese in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	CALCIUM	MAGNESIUM	ALUMINUM	MANGANESE	SULFUR	NITRATE NITROGEN
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	111	30	0	0	37	84
2	Calcium carbonate 2 gm.	270	13	0	0	50	128
3	Dried blood 4 gm.	255	54	0	34	58	294
4	Dried blood 4 gm., calcium carbonate 2 gm.	736	34	0	0	95	462
5	Dried blood 4 gm., calcium silicate 2 gm.	549	57	0	0	122	434
6	Dried blood, 4 gm., blast furnace slag 2 gm.	596	50	0	0	106	478
7	Ammonium sulfate 4 gm.	355	80	0	38	1,852	54
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	2,199	87	0	69	1,689	569
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	1,067	85	0	18	2,070	100
10	Sulfur 2 gm.	370	103	660	468	2,143	6
11	Sulfur 2 gm., calcium carbonate 4 gm.	858	108	68	297	1,294	35
12	Sulfur 2 gm., calcium carbonate 2 gm.	785	64	413	471	1,923	21
13	Sulfur 2 gm., dried blood 4 gm.	317	76	234	330	1,365	6
14	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	1,505	113	11	325	1,559	228
15	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	858	111	103	419	1,689	29
16	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	417	92	185	395	1,551	6

TABLE 8

Data for calcium, magnesium, aluminum and manganese in basic clay soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	CALCIUM	MAGNESIUM	ALUMINUM	MANGANESE	SULFUR	NITRATE NITROGEN
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	245	41	0	0	87	153
2	Dried blood 4 gm.	668	97	0	16	90	491
3	Ammonium sulfate 4 gm.	1,297	183	0	8	1,696	280
4	Sulfur 2 gm.	1,773	282	40	48	2,156	16
5	Sulfur 2 gm., dried blood 4 gm.	1,763	248	52	44	2,225	12
6	Sulfur 2 gm., ammonium sulfate 4 gm.	2,080	275	79	56	3,480	42

treatment. Where sulfur and dried blood were in contact, calcium was changed to a soluble form by a combination of reactions which resulted in the formation of ammonium sulfate. In these mixtures the basicity was not sufficient to neutralize the acidity produced, so that complete nitrification of

ammonia was prevented, and a part of the ammonia combined with sulfuric acid resulting from oxidation of sulfur.

Comparatively small amounts of magnesium were changed to a soluble state considering the total content of the soils. The silt loam soil contains approximately 2800 parts per million, slightly more than the total calcium; and the magnesium in the basic clay is 7000 parts per million. Where sulfur was not added to the silt loam, the soluble magnesium exceeds the aluminum and manganese found, but is decidedly less than the calcium brought into solution. In the solutions from mixtures where considerable acidity was produced by the oxidation of sulfur, some small increases are observed but they are not comparable with the much larger amounts of soluble manganese and aluminum.

Larger quantities of soluble magnesium were found in the solutions from the basic clay, which is in accord with the total magnesium of the soil. The concentration of magnesium in solutions for the clay soil was also greater than that of manganese and aluminum being the reverse of what was found for the silt loam soil.

ALUMINUM, IRON, AND MANGANESE

So far as evidence is furnished by the quantities of aluminum in the several solutions, the process of nitrification has had no solvent action on aluminum compounds in the two soils used in this experimental work.

No aluminum was extracted from mixtures which did not include sulfur as part of the treatment. The largest amount of aluminum sulfate was present in solution from soil where sulfur was oxidized in the absence of calcium carbonate.

The addition of calcium carbonate at the rate of 8000 parts per million of soil, decreased the solubility of aluminum to 68 parts per million, as compared with 660 parts per million, where basic calcium and magnesium compounds were sufficient to enter into combination with only a small part of the sulfuric acid.

Acidity resulting from the oxidation of sulfur in the basic clay reacted chiefly with calcium and magnesium. Much smaller amounts of manganese and aluminum sulfates were formed in this soil than in the silt loam.

Nitrification of dried blood had a slight effect on manganese in both soils. Ammonium sulfate also had a slight solvent action. Manganese, existing in the soil as oxides and decomposed silicates was freely attacked by the acidity resulting from oxidation of sulfur. The manganese found in the solution generally exceeds the aluminum. No indication was obtained that iron compounds were appreciably affected by the processes of nitrification and sulfofication.

SUMMARY

The indications obtained from this experimental work are as follows:

Nitrification of dried blood and oxidation of sulfur in soil mixtures increased the water-soluble potassium.

The liberation of potassium was brought about by salts formed, rather than by the direct action of acidity on insoluble potassium compounds. Calcium nitrate was present where nitrification was stimulated by calcium carbonate. Ammonium sulfate was formed, by the reaction between sulfuric acid and ammonia from dried blood, where a deficiency of basic calcium compounds restricted the nitrification of ammonia. Considerable amounts of calcium, aluminum and manganese sulfate were also present in certain mixtures.

Ammonium sulfate, independent of the ammonia being nitrified, affected the solubility of potassium.

Calcium naturally present in the soil as silicate, and in other combinations has been readily attacked by the acidity resulting from nitrification and sulfofication. Ammonium sulfate was also an active solvent of calcium.

Magnesium compounds were much more resistant to the action of these solvent agencies.

Large quantities of aluminum and manganese were converted into soluble forms where sulfur was oxidized in an acid silt loam soil. Small amounts of these elements were made soluble by this action in a basic clay. Nitrification had no effect on aluminum.

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NOTES ON RECENT WORK CONCERNING ACID SOILS

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In 1916 Gillespie (2) and the authors (8) showed by direct hydrogen electrode or colorimetric measurements that many soils are acid. This term signifies that the solutions of such soils have a greater concentration of H-ions than OH-ions. Subsequent work in other laboratories as well as in our own has substantiated this fact and has made clear the importance of recognizing and applying these simple principles of chemistry. Recently two articles have appeared in SOIL SCIENCE dealing with this subject on which the following brief comment is thought necessary, since it appears desirable to make every effort to bring about agreement on the chemical principles underlying soil problems.

Gillespie (3) has quoted from a previous article by the authors the expression "The rational treatment of acid soils requires that sufficient lime be added to bring the soil to a neutral or slightly alkaline reaction." This sentence when considered apart from the context of the several articles on acid soils gives an erroneous impression of our views. By the above expression, perhaps unhappily chosen, we have meant only to indicate that if one proceeded on the assumption, implied in so many of the so-called lime-requirement methods, that a neutral or slightly alkaline reaction is desirable for plant growth, then the most appropriate method would be to add lime to the soil in the quantities necessary to produce the desired reaction as measured by the hydrogen electrode. We did not intend to express any opinion with regard to the optimal reaction for the growth of plants, as evidenced by the fact that one of us (4, 5) has since investigated this point and has not found that an H-ion concentration comparable to that of most acid soils is inhibitive to the growth of many common plants of agricultural interest. Consequently, we have not been and are not now opposed to Gillespie's view that no arbitrary P_H value can be assigned as optimal for plant growth.

We are also in agreement with Gillespie in his statement that the attainment of a neutral reaction in the soil does not mean that all the water-soluble acids are neutralized. Our own experiments have in fact indicated that lime may be added to an acid soil until a slightly alkaline reaction is maintained for a number of days or weeks and that subsequently after a longer period of time an acid reaction reappears (though of a less concentration of H-ion than at the beginning). Theoretically it might be possible to add a sufficient quan-

tity of base to neutralize all acid substances present, but limitations of time, fineness of division of the soil, etc., would perhaps make such a neutralization impracticable. Finally, we desire to say that except to correct the impression noted above, our general viewpoint and method of attack are in entire accord with those of Dr. Gillespie.

The second article to which we wish to refer has been published recently by Rice and Osugi (7) in SOIL SCIENCE. This article deals with the inversion of cane sugar when heated in contact with soils. The data presented are very interesting but certain conclusions require a critical examination. In the first place, it is claimed in the course of their discussions that the determinations of H-ion concentration as carried out by Gillespie and by the authors may be affected by the carbon dioxide present and so lead to erroneous conclusions with respect to the nature of soil acidity. In reply to this we should like to

TABLE 1

Effect of boiling soil suspensions on PH *value*

SOIL NUMBER	SOIL SUSPENSION WITHOUT PREVIOUS HEATING	SOIL SUSPENSION AFTER BOILING*
	P_H	P_H
1	5.5	5.5
2	4.4	4.1
3	5.3	5.3
4	5.7	5.5
5	7.3	7.2

* The suspensions were boiled for ½ hour and then cooled while passing a rapid stream of hydrogen gas. The values given represent the average of several closely agreeing determinations.

call attention to one of our previous articles (6) in which specific data appear on this point. We have proved that even percentages of carbon dioxide as high as 9 per cent did not appreciably affect the reaction of acid soils. That the results would not be significantly changed by previous boiling of the suspension is evident from the determinations given in table 1.

In the experiments of Rice and Osugi it was found that many soils gave a very marked inversion of cane sugar while their extracts displayed scarcely any inverting power. Moreover the P_H values of the sugar extracts (determined by Sörensen's method) were in nearly every case practically neutral. Since the H-ion is considered to be the chief catalytic agency involved in the inversion of cane sugar, Rice and Osugi conclude that the so-called "true acidity" of soils is determinable only through the catalytic method and is generally the property of the insoluble portion of the soil. If the generally accepted belief be true that the catalysis of the inversion of sugar is a function of the H-ion concentration,[1] then it is obvious that the rate of inversion is first of all

[1] Acree, among others, has presented evidence supporting the view that non-ionized acids may also take part in the inversion of cane sugar. Acree, S. F., and Layng, T. E., 1913, Reaction of both ions and non-ionized forms of electrolytes. *In* Amer. Chem. Jour. v. 49, p. 345–368.)

dependent upon the solution, which is necessarily the only phase of such a system carrying H-ions. The H-ions, however, must be in equilibrium with the undissolved soil mass, although we do not understand the exact nature of this equilibrium. It is difficult to comprehend how the solid portion of the soil can effect inversion independently of the solution with which it is in contact. It is true that the solid phase exerts a considerable influence on the rate of inversion, but this must be attributed to modifications and readjustments of the solution effective for inversion as will be referred to later.

Since some of the explanations advanced by Rice and Osugi are at variance with our conceptions of these phenomena we conducted a number of experiments on the inverting power of soils and their extracts. The method used

TABLE 2

PH *values and inverting power of soils and soil extracts*

SOIL NO.	SOIL SUSPENSION	WATER EXTRACT OF SOIL*	WATER EXTRACT OF SOIL AFTER BOILING	SUGAR EXTRACT OF SOIL	SUGAR INVERTED BY WATER EXTRACTS OF SOILS: CALCIUM AS DEXTROSE PER 50 CC.	SUGAR INVERTED IN PRESENCE OF SOIL: CALCIUM AS DEXTROSE PER 50 CC.
	P_H	P_H	P_H	P_H	*gm.*	*gm.*
†15	3.4				0.163	0.268
12	4.5			4.2		0.147
6	4.4	4.4	4.6	4.4	0.050	0.114
2	4.3	4.4	5.0	3.8	0.069	0.122
5	4.6			4.8	0.030	0.071
13	5.6	5.3	5.5	5.1		0.026
14	5.9	5.1	5.6	5.6		0.025
10	5.4	5.1	5.1	5.7		0.010
11	6.3	5.7	5.5	6.2		0.004
7	7.5			6.8	0.003	0.014
8	7.5			6.5	0.001	0.009
9	7.5			6.8	0.001	0.002

* In a number of cases the water extract of the soil was too cloudy to make possible a satisfactory estimation of the H-ion concentration by the colorimetric method.

† Sample of silicic acid.

was the same as that proposed by Rice and Osugi; namely heating the 10 per cent sugar solution and the soil or extract for one hour at a temperature of 85°C. and then determining the amount of copper reduced. The results are summarized in table 2.

The first point to be noted is that the soils of highest H-ion concentration caused the greatest inversion and the very slightly acid soils and alkaline soils gave only a slight inversion. A very marked inversion was also produced by the extracts of the soils of H-ion concentration. It is unquestionably true that a larger inversion resulted when the sugar solution was heated in the presence of the solid soil particles. The data available at present do not enable us to explain satisfactorily why the presence of the solid particles should induce a more rapid rate of inversion than the solution by itself. Possibly a greater

concentration of H-ion is present in the films of solution closely associated with the soil particles, or it may be that H-ions are temporarily combined with carbohydrate molecules during the inversion and that the presence of the solid phase maintains a more constant concentration. It is to be remembered that the absolute quantity of H-ion in solution at any one time is very small and that only a small fraction of the total sugar is inverted. The above explanations are, of course, only conjectures which at present cannot be substantiated by direct evidence. But whatever may be the exact function of the solid material, it is fair to conclude from our data that large inversion occurs only in soils of distinctly acid reaction, with the greatest inversion occurring coincidently with the highest H-ion concentration of the soil suspension as well as of the water and the sugar extracts. The inversion of sugar and the subsequent reduction of copper are very complex processes in which other factors than the concentraion of H-ion may be important, so that when only slight quantities are inverted these are not necessarily to be correlated with the exact H-ion concentration.

The data of the above table also gave direct evidence that acid soils do give acid filtrates. The extracts were filtered through no. 590 S and S filter paper, and the H-ion concentration determined by the indicator method of Clark and Lubs (1). The filtrates gave acid reactions, generally of a magnitude very similar to those obtained with the suspensions. Boiling the extract for several minutes produced no large changes in H-ion concentration. Gillespie has previously shown that the colorimetric and hydrogen electrode determinations are usually in much closer agreement than the above when all suitable precautions are taken. It is difficult to understand why the statement is so often made that acid soils give alkaline extracts. If the latter are made with pure water and if the filtering medium is entirely inert, such a change of reaction can not occur. As pointed out in a previous paper (6) the reactions of boiled extracts made with water saturated with carbon dioxide have no bearing on the H-ion concentrations of the naturally existing soil solutions.

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THE EFFECT OF CERTAIN COLLOIDAL SUBSTANCES ON THE GROWTH OF WHEAT SEEDLINGS[1]

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The power of a soil to retain water against the force of gravity, to counteract the bad odors of decaying organic matter, and to modify the concentration of a solution with which it is in contact, is due wholly or in part to the phenomenon of absorption. Since the chemical composition of the soil is so complex it is difficult to ascertain to what extent the process of absorption from solution by the soil in a given case is chemical and to what extent it is physical. The absorption by some of the relatively insoluble soil constituents such as silica, and iron and aluminum oxides is probably physical and is termed adsorption. Within the last few years considerable work has been done pertaining to the absorption of salts and ions from solution by soils and other finely divided materials. Little attention, however, has been given to the effect this might have upon plant growth.

As a rule, adsorption is positive, that is, the concentration of a salt in the bulk of a solution is usually reduced by introducing absorbing surfaces into the solution. Consequently, the concentration of the salt at the interface is greater than that of the bulk. The object of this work was to attempt to answer the following question: Is the change in concentration due to solid adsorbing surfaces sufficient to modify the production of dry matter in a plant?

REVIEW OF LITERATURE

Gregoire (5) grew barley to maturity in Detimer's nutrient solution to which was added three-tenths of a per cent of silica in one treatment, and the same per cent of aluminum oxide in another treatment. Solution cultures were used as checks. There was a decided increase in the yield of dry matter in the silica cultures, and an increase in the case of the aluminum oxide cultures, but less than for the silica. More than 43.5 per cent of the ash of the plants from the silica culture consisted of silica, while less than 5 per cent of silica was present in the ash of the check. This author believes that the increase was due largely to the absorption of silica by the plants and its consequent utilization in growth. The ash of the plant in the alumi-

[1] A thesis submitted to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

num oxide culture contained 6 per cent alumina, while there was less than 1 per cent alumina in the ash from the check.

By growing wheat plants in solutions of several different densities, Lyon and Bizzell (8) showed that for the nutrient solution used the dry matter produced per unit of transpiration increased with the density. An increase in this ratio is considered indicative of greater density of the nutrient solution. They found that plants grown in crushed quartz had a lower transpiration ratio than when grown in nutrient solution of the same concentration. It appeared, therefore, that the actual densities in the region of the absorbing surface of the roots may be greater in the case of quartz. Plants grown in crushed quartz develop an abundance of root hairs and the root hairs come in intimate contact with quartz grains. It is possible, therefore, that the root hairs are in the absorbed layer, and are, therefore, feeding from a denser solution.

McCall (9) grew wheat plants in Shive's 3-salt solution when a solid medium representing somewhat the condition of the soil, but without the biological complications was present. He used a granite-ware pot provided with a pipe at the bottom for drainage. The solution was renewed every third day. The dry weights of the plants grown in the quartz sand were compared with those obtained by Shive. Sand cultures gave a greater dry weight. It is suggested by the author that the thickness of the adsorbed layer is less than the outer cell wall covering the adsorbing protoplasm, and since the absorbing protoplasm does not come in direct contact with this concentrated layer, the salts here are unavailable except in so far as the slow process of diffusion takes place.

It is also suggested in this article that selective adsorption may be an important factor in modifying plant growth. Thus the author calls attention to the fact that a better growth was produced in the sand than in the water cultures as the ratio of calcium nitrate to magnesium sulfate increased. This is explained by assuming that the NO_3 radicle produces a beneficial effect, that the magnesium sulfate antagonizes this effect, and that the magnesium sulfate more than the NO_3 would be adsorbed by the sand, and, therefore, less magnesium sulfate would be present in a condition to antagonize the favorable influence of the nitrate radicle. The results giving rise to this suggestion are subject to criticism since there is no record of the treatment having been duplicated.

Gile and Carrero (3) grew rice plants in acid, neutral and alkaline nutrient solutions when supplied with 0.002 gm. and 0.008 gm. of iron per liter from the following sources of iron: ferrous sulfate, ferric chloride, dialyzed iron, ferric citrate, ferric tartrate. The dialyzed iron was prepared by the ordinary method. It was entirely inadequate for the plants, for they were strongly chlorotic at all times.

Negeli (10), Dandeno (2), True and Oglevee (12, 13), Breazeale (1), Livingston (7), Jensen (6), and others have shown that the introduction of

finely divided substances like carbon black into nutrient solutions causes increased growth. This effect has been attributed to the action of the solid on certain organic and inorganic toxins.

True and Oglevee (13) advanced the theory that the adsorbing substances increase the growth rate by reducing the concentration of the toxic material, acting in the same way as the addition of water would by leaving a fewer number of ions or molecules in the free solution.

According to this theory it would be possible to introduce a solid substance into a nutrient solution and reduce the actual concentration of the solution in the region of the absorbing surface. If the theory is correct, this application is true, it being only necessary to select the proper adsorbent.

Dandeno (2) suggests that the increase in growth may be due to a decrease in the diffusion rate, while Jensen (6) adds a third possibility: viz., chemical change induced by the presence of the finely divided material.

The literature fails to give conclusive evidence as to whether the introduction of a solid adsorbent into a nutrient solution increases or decreases the density of that solution at the absorbing surface of the roots. The present work was intended as a contribution to this subject.

PLAN OF THE INVESTIGATION

Baker's analyzed salts were used in making up the nutrient solution. The salts were weighed and dissolved in distilled water, giving a stock solution for each salt. The following table shows the concentration and composition of each stock solution:

Calcium nitrate	216 gm. in 2000 cc. of water
Potassium chloride	60 gm. in 2000 cc. of water
Magnesium sulfate	48 gm. in 2000 cc. of water
Ferric sulfate	4 gm. in 1000 cc. of water
Mono potassium phosphate	60 gm. in 1000 cc. of water

A complete nutrient solution containing about 4500 parts per million was made up by adding 25 cc. of each solution to distilled water, as follows: The calcium nitrate and potassium chloride solutions were added to 250 cc. of distilled water. The ferric sulfate, magnesium sulfate and potassium phosphate solutions were diluted each with 175 cc. of distilled water and put in 3 different containers. The diluted ferric sulfate and magnesium sulfate solutions were mixed and then added to the potassium chloride and calcium nitrate mixture. Finally, the potassium phosphate solution was added and the mixture shaken. At the latter addition there was always a slight precipitation. The solution was not filtered but was always shaken thoroughly before using.

Galgalos wheat was used. The method of germinating and growing the wheat up to the point of placing it under treatment was the same in all cases and was as follows: A few hundred clean seeds were placed in a wide-mouth bottle provided with a cork with one hole through which a piece of glass

tubing was inserted, one end reaching to the bottom of the bottle. The other end was connected with the water tap. By providing a cork too large or slightly too small, the water would run over the top without allowing the seeds to pass out. The seeds were always kept in running water for 48 hours. They were then transferred to a large floating disc and here kept in running water until four or five centimeters in height. This required about four days. The floating disc was a piece of stiff wire netting covered with paraffin. Many small holes were made in the paraffin to furnish a contact between the seedlings and water. Many more plants were grown to this stage than were required for the experiment, which made more easy a selection of plants of uniform top and root growth. Strong healthy plants were always selected for the cultures. The endosperms were carefully removed and 6 plants threaded into holes in a cork that would fit the container to be used. The threading was most easily done by carefully pressing the tops together and passing them into the hole, and then working the seedling into the desired position. Each cork with the 6 plants was then placed in the container filled with tap water. It appeared early in the work that the placing of the plants under treatment as soon as the endosperm was removed was not satisfactory, for many of the plants died under these conditions, while if kept in tap water for a short time they developed very well. Four small sticks placed in holes bored near the circumference of the cork and tied together by twine furnished support for the tops of the growing plant.

Wherever possible the distilled water which was to be used in culture work was treated either with carbon black or with precipitated oxide of iron or aluminum. This treatment of the distilled water consisted in adding 15 gm. of the material to a liter of water, shaking and allowing to stand for a few hours and filtering.

The hydroxides of iron and aluminum were prepared from the chlorides of these elements, by precipitation from solution by NH_4OH and then washing free of ammonium chloride. The iron hydroxide was used at the rate of 10 gm. of dry material calculated as Fe_2O_3 and the aluminum hydroxide at the rate of 8 gm. Al_2O_3 per liter of distilled water. They were kept moist and used in the same way as the carbon black. In the following discussion the term "treated" nutrient solution has reference to the distilled water used.

During the period that plants were growing under treatment, they were kept in the greenhouse at a temperature of about 55°F. during the night and 60°F. during the day.

At harvest the tops were removed and dried at a temperature of 90°C. In order to obviate lack of uniformity resulting from an unequal distribution of heat in the greenhouse, the relative positions of the cultures were changed at each weighing. The cultures were weighed or the solutions were renewed twice each week.

The following materials, all of which have been found by different investigators to adsorb from solution, were used in the present work: agar, silica, crushed quartz, and hydroxides of iron and aluminum.

Agar cultures

The agar solution was prepared by adding to the dry agar sufficient distilled water to make a 2 per cent solution and boiling the mixture in a double boiler for 2 hours. The resulting solution was then cooled to a temperature of 60°–75°C., and the proper amount of standard nutrient solution and distilled water added to give the desired dilution of nutrient salts. In the earlier part of the work a one per cent solution of agar was used, but difficulty was experienced in getting the tender roots of the wheat plants to penetrate this medium. It was found more satisfactory to use one-half per cent agar as the plant roots would easily penetrate this medium. After the agar medium had cooled to laboratory temperature the plants which had previously been selected and threaded into the corks were placed in the agar. Water cultures of the same concentration of nutrient salts were prepared as checks, the plants being placed in the water and agar cultures at approximately the same time. In certain of the cultures the solutions were not renewed but each culture was weighed twice each week and water added equivalent to the loss in weight.

In other cases the solutions were renewed by lifting the cork carrying the seedlings, discarding the old medium, rinsing the container, and adding 300 cc. fresh medium from stock solutions of the proper strength. The results of these experiments are given in tables 1, 2 and 3.

The results with agar were not entirely satisfactory, but the figures present some interesting indications. Referring to table 1, it will be seen that in concentrations of 500 p.p.m. of nutrient salts, agar was beneficial, while with a concentration of 2000 p.p.m. the agar was harmful. A similar effect may be noticed in table 2, where in the 500 p.p.m. solution, agar was beneficial while in the 1000 p.p.m. solution, agar was harmful. With the exception of the 85 p.p.m. concentration, more dilute solutions were benefited by agar, while the higher concentrations were injured. It is conceivable that with the more concentrated solutions the agar rendered the effective concentration at the surface of the particles so great as to become toxic to plants.

TABLE 1

Dry weights of wheat plants grown in solution and in agar cultures. Solution not renewed; volume of solution 930 cc.; each result the average of 4 cultures

TREATMENT	CONCENTRATION	PERIOD OF GROWTH	DRY WEIGHT			DIFFERENCE	
			Tops	Roots	Whole plant		
	p.p.m.	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Nutrient solution + 1 per cent of agar	500	30	0.3240	0.1262	0.4502	+0.1740	62.9
Nutrient solution	500	30	0.1799	0.0963	0.2762		
Nutrient solution + 1 per cent of agar	2000	37	0.3011	0.1133	0.4144	−0.1098	−24.0
Nutrient solution	2000	37	0.3568	0.1674	0.5242		

Dry weights of wheat plants grown in a nutrient solution and in a nutrient solution with 1 per cent of agar. Volume of solution 500 cc.; solution not renewed; each result the average of 4 cultures

TREATMENT	CONCENTRATION	PERIOD OF GROWTH	WEIGHT OF			DIFFERENCE	
			Tops	Roots	Whole plant		
	p.p.m.	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Distilled water		18	0.0918	0.0311	0.1229		
Distilled water + agar		18	0.1239	0.0524	0.1763	+0.0534	+42.6
Nutrient solution	85	20	0.1643	0.0488	0.2252		
Nutrient solution + agar	85	20	0.1494	0.0478	0.1972	−0.0280	−12.4
Nutrient solution	250	29	0.2367	0.0604	0.2971		
Nutrient solution + agar	250	29	0.2790	0.0667	0.3457	+0.0486	+16.3
Nutrient solution	500	36	0.2721	0.0569	0.3290		
Nutrient solution + agar	500	36	0.3217	0.0681	0.3898	+0.0608	+18.1
Nutrient solution	1000	36	0.4420	0.1076	0.5496		
Nutrient solution + agar	1000	36	0.3075	0.0636	0.3711	−0.1786	−32.4

+ Indicates increase in agar culture compared with check.
− Indicates decrease in agar culture compared with check.

TABLE 3

Dry weights of wheat plants when grown for 5 weeks in a nutrient solution and in a nutrient solution to which was added 0.5 per cent agar. Solution renewed twice per week; volume of solution 300 cc.; concentration 750 p.p.m.

TREATMENT	LAB. NO.	WEIGHT OF		
		Tops	Roots	Whole plant
		gm.	*gm.*	*gm.*
Nutrient solution	80	0.4042	0.0905	0.4947
	81	0.5175	0.1133	0.6308
	82	0.4355	0.0858	0.5213
	83	0.6204	0.1401	0.7605
	84	0.5456	0.1198	0.6654
	85	0.5479	0.1479	0.6958
	86	0.4456	0.0839	0.5295
	87	0.5531	0.1179	0.6710
	88	0.5372	0.1239	0.6611
	89	0.4485	0.0923	0.5408
Average		0.5056	0.1114	0.6171
Nutrient solution + 0.5 per cent of agar	90	0.3951	0.0884	0.4845
	91	0.3786	0.0677	0.4363
	92	0.5104	0.0921	0.6025
	93	0.3970	0.0896	0.4866
	94	0.4119	0.0945	0.5064
	95	0.3629	0.0882	0.4511
	96	0.4551	0.0816	0.5373
	97	0.7340	0.1569	0.8909
	98	0.6323	0.1440	0.7763
	99	0.5562	0.0927	0.6489
Average		0.4829	0.0995	0.5824

Difference between the solution and agar cultures, −0.0347 gm. or − 5.62 per cent.

Silica cultures

Colloidal silica was prepared by dialysis following in general the method used by Graham (4). A preliminary test was made as follows:

With a pipette approximately 10 cc. of a 10 per cent solution of hydrochloric acid were measured into an Erlenmeyer flask. Sodium silicate (a 10 per cent solution) was slowly added from a burette while the mixture was shaken vigorously. As the sodium silicate increased, the mixture gradually became more viscous and finally set to a gel. The amount of silicate solutions required to form the gel was then noted. In preparing a quantity of the mixture for dialysis, only one-half to two-thirds of the total required sodium silicate solution was used. In a mixture of these proportions the silica will not set until the chlorides are reduced to a very small percentage.

Two kinds of dialyzers were used. A very efficient dialyzer was made by taking 5 feet of a heavy quality of parchment paper tubing, tying one end carefully and then telescoping it into a piece of glass tubing of a little less length but of somewhat greater diameter. The end of the glass tube containing the closed end of the parchment paper tubing was provided with a one-hole stopper and a short piece of glass tubing sealed into the hole in this stopper. One end of a rubber tube was then attached to the small glass tubing, the other end leading to a container of distilled water which furnished a head of three or more feet. The parchment paper was always soaked for two or three days in distilled water, and carefully tested for leaks. The solution to be dialyzed was run into the parchment paper tubing from the open end which was then tied and crowded into the glass tube. This end of the dialyzer, which was supported about two feet above the opposite end, was provided with a one-hole stopper and glass tubing for drainage to the sink. A constant stream of distilled water was then forced from the lower to the upper end, flowing outside of the parchment tubing and carrying the chlorides which diffused through the paper into the drainage. Such an arrangement exposes a large surface to a steady stream of water and therefore makes a very efficient dialyzer. As we were unable to obtain a sufficient amount of the parchment paper tubing for all the work with silica, a modified Stern dialyzer was also used. This consists of two pans each of about five liters capacity arranged so that a stream of distilled water comes in at the bottom of one, fills the pan and overflows evenly at the top. The other pan has parchment paper drawn tightly over the top and tied, and a hole made in the bottom large enough to insert a funnel. This pan is placed upon the first pan bottom up so that the parchment paper comes in contact with the water in the first pan. Dialysis begins at once when the mixture of sodium silicate and hydrochloric acid is put in the top pan. A few short pieces of filter paper laid between the two pans help to establish a uniform distribution of the water as it flows from the pan. About five liters could be dialyzed at one time with this apparatus which requires a period of nearly three weeks

with the water running continuously. As dialysis continues, the viscosity of the sol gradually increases, finally setting to a gel. The extent to which the silica can be purified before setting depends, of course, upon the concentration of the silica. Thus in one case with 2.8 per cent of silica, gelation began when nearly 5000 p.p.m. of chlorides were present, while in another case with 1.5 per cent of silica, gelation did not begin until the chloride content was reduced to 700 p.p.m. The silica was never allowed to set firmly in the dialyzer, but was siphoned into a large container and then enough measured into the culture jars to give 1 per cent of silica when diluted to 500 cc. The dilutions were made from the standard nutrient solution or from this solution and distilled water. Before measuring the colloidal silica solution into the jars for the cultures, total solids and chlorides were determined and the latter calculated as sodium chloride. As it was not practicable to remove all chlorides from the silica, sodium chloride was added to the cultures containing no silica so that all culture media contained the same amount of

TABLE 4

Dry weights of wheat seedlings grown for 27 days in pure nutrient solutions and in nutrient solutions containing 1 per cent silica

TREATMENT	DRY WEIGHT OF TOPS	INCREASE DUE TO SILICA	
	gm.	*gm.*	*per cent*
Nutrient solution 85 p.p.m.	0.3627		
Nutrient solution 85 p.p.m. + silica 1 per cent.	0.4276	0.0649	17.8
Nutrient solution 250 p.p.m.	0.4594		
Nutrient solution 250 p.p.m. + silica 1 per cent.	0.5936	0.1342	29.2

sodium chloride. Wheat plants were selected, threaded into corks and transferred from tap water to the culture solutions in the manner described for agar cultures. Within two to three days the plants growing in nutrient solutions containing 1 per cent of silica were larger and had better color than those growing in pure nutrient solutions. This difference was maintained until harvest. The results of this experiment are given in table 4.

The weights of roots are not given in the above table since it was not always possible to make an accurate separation from the adhering gel.

The figures in table 4 show that the silica was decidedly beneficial to the wheat seedlings. It occurred to the author that the effect might be due to increased absorption of silica by the plant. Determinations of silica in the seedlings showed that the suspicion was well founded. The data may be seen by reference to table 5. An attempt was made to obviate this disturbing factor by introducing into one set of cultures 50 p.p.m. of colloidal silica, on the assumption that this amount would supply the requirements of the plant for silicon. Accordingly, three sets of cultures were set up in the manner already described. Four concentrations of nutrient salts were used. The data are given in table 5.

TABLE 5

Dry weights and silica content of wheat seedlings grown in nutrient solutions and in nutrient solutions containing silica

TREATMENT	DRY WEIGHT OF TOPS	SILICA IN DRY MATTER
	gm.	*per cent*
Nutrient solution 85 p.p.m.	0.2628	5.3
Nutrient solution 85 p.p.m. + colloidal silica 50 p.p.m.	0.2875	8.3
Nutrient solution 85 p.p.m. + silica gel 1 per cent.	0.4669	16.0
Nutrient 250 p.p.m.	0.5635	3.1
Nutrient solution 250 p.p.m. + colloidal silica 50 p.p.m.	0.5846	5.1
Nutrient solution 250 p.p.m. + silica gel 1 per cent.	1.0540	20.2
Nutrient solution 500 p.p.m.	0.5743	2.8
Nutrient solution 500 p.p.m. + colloidal silica 50 p.p.m.	0.6389	3.9
Nutrient solution 500 p.p.m. + silica gel 1 per cent.	1.1404	22.8
Nutrient solution 1000 p.p.m.	0.9688	2.0
Nutrient solution 1000 p.p.m. + colloidal silica 50 p.p.m.	0.7961	3.6
Nutrient solution 1000 p.p.m. + silica gel 1 per cent.	1.4235	31.3

The results show a striking correlation between silica in the culture medium, growth of plant, and silica in plant. The medium does not, therefore, fulfill the requirement of an inert colloidal substance and is, therefore, not suitable for the problem undertaken in this study. The data are given merely for the benefit of those who may wish to undertake similar work.

Sand cultures

The amount of salt adsorbed from a solution by an adsorbent depends upon the surface exposed by the adsorpent. If a change in the concentration of the nutrient salts due to adsorption is sufficient to modify the production of dry matter in plants, the difference ought to be increased when the fineness of the adsorbent is increased. Quartz sand was selected as the adsorbent on the assumption that being very insoluble it would not supply nutrients to the plant. Three different grades of sand were secured from the New England Quartz Company of New York City. These are known commercially as no. 000, 1 and 2. A mechanical analysis is given in table 6.

The sands were purified by heating with hydrochloric acid (1: 3) for about ten hours, washing with distilled water until free from chlorides, thoroughly drying and storing for future use.

In growing plants either in soil or in water cultures it is necessary to replace the water lost by transpiration and evaporation. With water cultures this renewal is accomplished either by adding at frequent intervals sufficient distilled water to maintain the original volume or by discarding the old medium entirely and adding fresh nutrient solution equivalent to the original amount of the old. The second procedure is preferable since it eliminates the possible accumulation of toxic excreta. With quartz sand cultures renewal of solutions has not been practiced. The method used by McCall

in which solutions were renewed but old sand retained did not take into account the possible saturated condition of the quartz after continual use. The writer has devised a method for renewing not only the nutrient solutions but also the quartz.

For this purpose glass fruit jars of 1 pint capacity were used. A hole $\frac{1}{2}$ inch in diameter was drilled in the bottom of each jar. The holes were stoppered and plants which had been selected and threaded into the corks were transferred to the jars which were to be used in the experiment. A volume of 250 or 300 cc. of nutrient solution of the desired concentration was transferred to the jar by means of a funnel, a large hole having previously been made in the cork for this purpose. Seven hundred grams of quartz sand was then weighed, a large funnel inserted in the hole of the stopper, and the sand allowed to fall gently into the solution. By using a small scoop and

TABLE 6

Mechanical analysis of quartz sands

	NUMBER 2	NUMBER 1	NUMBER 000
	per cent	*per cent*	*per cent*
Fine gravel (2–1 mm.)	0.08	0	1.53
Coarse sand (1.0–0.5 mm.)	21.09	0	2.63
Medium sand (0.5–0.25 mm.)	68.93	2.25	8.93
Fine sand (0.25–0.1 mm.)	8.45	92.51	69.62
Very fine sand (0.1–0.05 mm.)	0.16	1.91	12.27
Silt and clay (less than 0.05 mm.)	0.86	3.29	4.78
	99.57	99.96	99.76

sprinkling the sand into the funnel the stream of sand falling into the solution could easily be regulated. The roots of the plants were completely buried in the sand. At renewal periods the old sand and solution were removed by first taking out the small stopper at the bottom of the container, and then allowing a gentle stream of distilled water to run in at the top, the cork holding the plants remaining in place. Being under a low head this water never ran swiftly. A large glass siphon was used, and to give flexibility to that end which was inserted through the hole in the stopper containing the plants, a piece of rubber tubing was attached. The fresh solution and sand were added in the same manner as the first addition.

All solutions were renewed twice per week. The checks were solution cultures and were grown in the same amount and same concentration of nutrient solution as the sand cultures. The distilled water used in all cultures was treated with carbon black. The process of renewal just described appears not to be detrimental to the seedlings, as the comparison in table 7 will show.

TABLE 7

Dry weights of wheat seedlings grown in quartz to which nutrient solutions were added

	WEIGHTS OF SEEDLINGS GROWN IN	
	Quartz sand number 2	Quartz sand number 000
	gm.	*gm.*
Nutrient solution and sand renewed twice each week.....	0.6551	0.8033
Nutrient solution and sand not renewed, but distilled water added.....	0.5089	0.5888

* These figures indicate a better development where solutions were renewed.

In tables 8 and 9 are given the results of experiments showing the effect of quartz sands of different degrees of fineness on the growth of wheat.

TABLE 8

Dry weights of wheat plants grown 35 days in nutrient solutions with and without quartz sand of different degrees of fineness.

(*Concentration of nutrient solution 750 p.p.m.; sand and nutrient solution changed twice per week.*)

TREATMENT	DRY WEIGHT OF WHEAT SEEDLINGS. TOPS ONLY						AVERAGE
	1	2	3	4	5	6	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nutrient solution.....	0.2624	0.2717	0.2011	0.2816	0.2449	0.2339	0.2492
Nutrient solution + sand no. 2.....	0.2158	0.2440	0.2362	0.2448	0.1920	0.2663	0.2332
Nutrient solution + sand no. 1.....	0.2382	0.2245	0.2279	0.2280	0.2368	0.2312	0.2311
Nutrient solution + sand no. 000.....	0.1911	0.2298	0.2179	0.2356	0.1984	0.1842	0.2073

TABLE 9

Dry weights of wheat plants grown for 28 days in nutrient solutions with and without quartz sand of different degrees of fineness.

(*Concentration of nutrient solution 370 p.p.m.; sand and nutrient solution renewed twice each week.*)

TREATMENT	DRY WEIGHT OF WHEAT SEEDLINGS. TOPS ONLY					AVERAGE
	1	2	3	4	5	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nutrient solution.....	1.0442	1.1595	1.0149	1.2198	1.1670	1.1211
Nutrient solution + sand no. 2..	0.6827	0.6175	0.6871	0.6696	0.6038	0.6551
Nutrient solution + sand no. 000.	0.8749	0.8826	0.8930	0.6370	0.8743	0.8033

It will be seen that the amount of dry matter produced is greater for a nutrient solution of a given concentration than for the same concentration in sand cultures. Although the differences are small, it seems sufficient to indicate that the decrease in concentration of the nutrient solution by the sand may be a factor causing a smaller production of dry matter.

Ferric hydroxide cultures

It occurred to the author that hydroxides of iron and aluminum might be suitable for a study of adsorption. These substances are relatively insoluble, are colloidal in character, and supposedly would not furnish nutrients to increase plant growth. The ferric hydroxide was prepared as follows: A 10 per cent solution of chemically pure ferric chloride was prepared and placed in a large bottle. Ammonium hydroxide was then added carefully until the mixture was faintly alkaline to litmus. The bottle was then filled with distilled water, stoppered, shaken thoroughly, and allowed to stand until the precipitate had settled. The supernatant liquid, was siphoned off and the washings repeated until free from chlorides. After the last washing the suspension was allowed to settle for several weeks and the supernatant liquid drawn off. A sample of the remaining suspension was used for determination of the ferric hydroxide and the remainder used in the cultures to be described. The cultures were made up so that all solutions contained the same concentration when calculated on a basis of total volume of water and total nutrient salts. Sufficient ferric hydroxide suspension was used to give 1.5 per cent ferric hydroxide calculated on volume of culture solution. The plants were grown in glass tumblers of 300 cc. capacity, the plants being previously selected and threaded into corks and placed in the different cultures. In all cases the entire medium was renewed twice each week. The results of the experiment are given in table 10.

No difference could be seen in the general appearance of the ferric hydroxide cultures and the checks during the early part of the growing period, except that the latter in most cases developed a heavier growth. Towards the end of the growing period, however, the plants in the ferric hydroxide cultures had a somewhat darker green color. The duration of the growing period was in every case determined by the plants in the iron, there being a more marked yellowing of the ends of the leaves in these cultures. These effects may result from an excessive use of iron in the metabolism of the plant, although, in view of the work of Gile and Carrero and from the insolubility of the colloidal iron, this would not seem to be the case.

In every case the dry weight of the tops is less for the plants grown in ferric hydroxide cultures.

The colloidal ferric hydroxide adheres to the roots forming a very tenacious covering. The roots were always washed carefully in distilled water before drying, but it was impossible to remove all of the iron oxide from them.

TABLE 10

Dry weights of wheat plants grown in nutrient solution and in nutrient solution containing ferric hydroxide calculated as Fe_2O_3

TREATMENT	WEIGHT OF TOPS ONLY	AVERAGE
Length of growing period—5 weeks		
	gm.	*gm.*
Nutrient solution, 750 p.p.m.	0.4042	
	0.5175	
	0.4355	
	0.6204	
	0.5456	
	0.5479	
	0.4456	
	0.5531	
	0.5372	
	0.4485	0.5056
Nutrient solution 750 p.p.m. + ferric hydroxide 1.5 per cent	0.3706	
	0.3283	
	0.4044	
	0.5868	
	0.4408	
	0.3499	
	0.3507	
	0.3457	
	0.4374	
	0.3876	0.4002
Length of growing period—30 days		
Nutrient solution 750 p.p.m.	1.2430	
	1.7361	
	1.9641	
	1.6378	
	1.6540	1.6490
Nutrient solution 750 p.p.m. + ferric hydroxide 1.5 per cent	0.8435	
	1.1832	
	0.7775	
	0.7405	
	0.9629	0.9015
Length of growing period—31 days		
Nutrient solution 370 p.p.m.	1.1147	
	1.3210	
	1.6747	
	1.5611	
	1.5614	
	1.4955	1.4894
Nutrient solution 370 p.p.m. + ferric hydroxide 1.5 per cent	0.9688	
	0.9892	
	1.1689	
	1.0984	
	1.0029	
	1.0927	1.0535

Aluminum hydroxide cultures

The procedure already described for the preparation of ferric hydroxide was followed in preparing the aluminum hydroxide, the chloride of aluminum being used in the place of ferric chloride. The suspension was made of such strength that the cultures contained 2 per cent of aluminum hydroxide calculated as Al_2O_3. The experiment was performed at the same time and under the same conditions as were the ferric hydroxide cultures. The results are given in table 11.

TABLE 11

Dry weights of wheat plants grown in nutrient solution and in a nutrient solution containing 2 per cent of aluminum hydroxide calculated as Al_2O_3

TREATMENT	WEIGHT OF TOPS ONLY	
Length of growing period—31 days		
	gm.	*gm.*
Nutrient solution 370 p.p.m.	1.1147	
	1.3210	
	1.6747	
	1.5611	
	1.5614	
	1.4955	1.4894
Nutrient solution 370 p.p.m. + aluminum hydroxide 2 per cent.	1.1188	
	1.4504	
	1.2624	
	1.1612	1.2481
Nutrient solution 750 p.p.m.	1.6490	1.6490
Nutrient solution 750 p.p.m. treatment with Al_2O_3..	1.3607	
	1.4984	
	1.3131	
	1.5164	1.4222
Nutrient solution 750 p.p.m. + aluminum hydroxide 2 per cent.	1.4411	
	1.6344	
	1.1921	
	1.7367	
	1.5414	1.5091

Difference between nutrient solution (treated) and nutrient solution with Al_2O_3 gm. — 0.0530, per cent — 2.8.

The results in table 11 show that aluminum hydroxide is similar to ferric hydroxide in that its addition to nutrient solutions caused a decrease in the growth of the wheat plants.

SUMMARY

The effect of adding agar to nutrient solutions was to increase the growth of wheat seedlings in low concentrations and to decrease the growth in higher concentrations of nutrient salts. The introduction of colloidal silica into nutrient solutions resulted in increased weight of wheat seedlings. The increase was apparently due to direct absorption of silica by the plant and not to a change in the effective concentration of the nutrient solution. Silica gel is, therefore, considered unsuited for studies of the character described in this paper. The introduction of quartzs and, ferric hydroxide, and aluminum hydroxide into nutrient solutions resulted in decreased growth of wheat seedlings. It appears that these substances by their absorptive properties reduce the effective concentration of the nutrient solution.

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THE LONGEVITY OF B. RADICICOLA ON LEGUME SEEDS

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As a practical problem, the longevity of the nitrogen-gathering bactera upon the coats of legume seeds, is an important one. Since inoculation of legumes is so widely practiced, and of the several methods in vogue, probably the seed inoculation method is the one most generally used, it is desirable to know the best inoculant to use as well as the most efficient and economical way of applying it.

Oftentimes the farmer inoculates his seed, and on account of rain, or for other reasons, he cannot plant it at once. The question immediately arises—How long can enough bacteria survive on these seeds to produce good inoculation of the plants? Obviously this time varies with climatic conditions, methods of applying the bacteria to the seeds and the variety of legume. Other factors may also cause bacteria to perish soon after inoculation.

The idea was conceived that if the bacteria survived on the seedcoats of the common legumes for a sufficient length of time, then the seed dealers could attend to the inoculation of seed and thus save the planter the time and trouble, besides carrying it out in a more uniform and economical manner. Most large dealers in seeds are also dealers in legume cultures and for this reason would be able to do the work cheaply. Many of the failures in alfalfa growing may be attributed to ignorance of the necessity of inoculation. If all seed could be inoculated before planting, better results in the growing of certain legumes for the first time would be obtained. Many farmers have an antipathy against handling bacteria, a term which implies and is often considered synomonous with disease. If legume seeds could come ready to plant, it would also further their introduction into certain localities and thus be of great economic gain to the country.

Very few experiments have been carried out on this question, and these are not exhaustive enough to draw any practical deductions from. Temple (7), of the Georgia Agricultural Experiment Station, has shown that *Bacillus radicicola* may live for months in dry soil and on the seedcoats of cowpeas. It is a well known fact that both the symbiotic, as well as the non-symbiotic, nitrogen-fixing bacteria may live for years in dry soil. The writer found that dry soil, taken from a soybean field 18 months before, caused copious nodule production on soybean plants, which were grown in uninoculated soil. Plants grown in check pots, side by side, showed no inoculation. Thus it

has been already established to a certain extent that the nodule organisms can, under certain conditions, withstand considerable desiccation.

The cotton cultures, of the U. S. Department of Agriculture (5) failed, because the bacteria soon died on the dried strands of cotton. Chester (1) in 1907 described some experiments showing extremely variable results regarding the desiccation of root tubercle bacteria. His work consisted in the main of depositing pure cultures of *B. radicicola* on cotton gauze or wool, drying at 35°C. (a temperature far above that to which this organism is commonly exposed and at which it nearly always fails to grow on agar plates), and testing for viability at fixed periods after the drying was complete. He found that the cotton became sterile at periods varying from a few hours to 62 hours in one case. He states, "*Ps. radicicola* has little power to withstand drying and its preservation in the dry state on cotton is inadvisable". This organism also died quickly when deposited upon glass in thin films. As a result of a short experiment with alfalfa seed, he states, "*Ps. radicicola* when dried on seed perish very quickly".

Noyes and Cromer (6) made tests to determine the amount of infected soil or commercial cultures necessary to produce inoculation. The main criticism with this work is that the amounts used per acre were much too small. The writer (3, 4) showed that commerical cultures for legume inoculation vary greatly in quality. Of all the cultures tested, the muck and soil cultures gave the best results. He also showed that certain seeds, like the soybean, were very difficult to inoculate. Several of the cultures tested contained sufficient bacteria to give 100 cells per seed under ordinary conditions of planting. It is very desirable to know just how long these cells will remain alive and active on the seedcoat of the legume before the seed is planted or after it has been planted in dry soil. For this reason, the following series of experiments have been carried out.

EXPERIMENTAL

It was first necessary to determine a satisfactory method of sterilizing the seeds, in order to insure reliable results from the experiments. Several well known substances were tried in this work and a comparison test made. Soybean seed, of the Black Eyebrow variety, was used in all of the following tests.

A. Formalin

First the seeds were soaked in a 1 per cent formalin (40 per cent formaldehyde), solution for 15 minutes, rinsed in five washings of sterile water and placed aseptically in tubes of beef broth, one seed to the tube. After 48 hours but 20 per cent of the tubes showed no growth. The germination of the seed was reduced from 86.5 to 56.0 per cent. Solutions of formalin, varying in strength from 1 to 5 per cent, were next tried in the same manner, except that the time of immersion of the seeds was reduced to 3 minutes. In

no case was sterilization complete, although in the case of the 4 per cent and 5 per cent solutions 60 and 80 per cent, respectively, of the tubes were sterile. In the second series, solutions of formalin ranging from 1 to 5 per cent were allowed to act upon the seeds for 10 minutes. Here again germination was seriously interfered with. It was found that seeds treated with a 4 or 5 per cent formalin solution for 10 minutes were usually free from bacteria, but still retained viable fungus spores.

B. Hydrogen peroxide

Acting upon a suggestion of Dr. John W. Shive, plant physiologist of the New Jersey Agricultural Experiment Station, hydrogen peroxide was used to treat the seeds. They were soaked in a 2 per cent solution for 5 minutes. Only a small part of the seeds were found to be sterile, but instead of injuring germination, it was greatly enhanced and the early growth of the seeds was much stimulated. This may have been due to the action of the liberated oxygen upon the seeds. The method was not tried further.

C. Calcium hypochloride

Ordinary bleach showing on analysis 33 per cent available chlorine was used in such dilution as to give a solution containing approximately 6 per cent chlorine. The seeds were immersed for periods of 5 and 10 minutes. This chemical gave good results and did not materially affect germination. From 80 to 100 per cent of the tubes containing treated seeds were found sterile after 48 hours.

D. Mercuric bichloride

The seeds were placed in a 1 to 500 solution of mercuric bichloride for 5 minutes, removed aseptically and washed in 5 changes of sterile distilled water. The whole time occupied about 10 minutes. Upon testing these seeds in bouillon tubes, practically all (95 per cent) were sterile. Germination was somewhat reduced, but this was not a vital factor in this experiment, as twice as many seeds were planted as were allowed to grow to maturity. The qualitative analysis of 5 of the treated seeds showed only the merest trace of mercury. It is not believed that this would exert a toxic effect upon the bacteria to be added later to the seeds.

Treatment with bichloride of mercury gave such satisfactory results that it was finally adopted in the work. Probably bleach is just as good, as Wilson (8) has shown, but the writer began the use of the mercury salt and continued it throughout the experiments.

MEDIA USED IN THE WORK

A comparison of several media was made, the results being reported elsewhere (3). A modified Ashby's medium was found to give the best all round results besides being easily made up. This medium contains mannite, 10

gm., mono-potassium phosphate 0.2 gm., magnesium sulfate 0.2 gm., calcium sulfate 0.1 gm., calcium carbonate 1.0 gm. These salts were dissolved in 1,000 cc. of distilled water, to which 12 gm. of agar had been added. The agar was purified previous to use by soaking in water for 6 hours to remove soluble substances. The mono-potassium phosphate was neutralized separately with normal potassium hydrate. While testing commerical cultures of legume bacteria sometime previous to carrying on this experiment, a medium was used containing 15 gm. of agar and 12 gm. of mannite per liter, in addition to the above salts. It is believed, however, that the smaller amounts of mannite and agar are preferable, for on comparing, the media colonies of *B. radicicola* were larger and more characteristic. This is in accord with previous observations of the writer (2).

Soybean seed of the Black Eyebrow variety were used in the main experiment, although some seeds of the Early Brown variety were used in the preliminary experiment. These seeds had been previously treated with bichloride of mercury and sterile water and had been dried between sterile blotting pads in a quiet, dark room. The inoculum was prepared by crushing 25 gm. of fresh, thoroughly washed soybean nodules in 500 cc. of sterile water. This infusion was then filtered through several layers of cheese cloth. The seeds were placed in this filtered nodule infusion for the following periods of time: 2 minutes, 20 minutes, 6 hours and 24 hours. After this they were removed, and placed on sterile blotting paper in a quiet, dry, dark room for several hours. On becoming dry, the seeds were transferred aseptically to sterile paper bags and stored away. The first seeds were tested bacteriologically, as well as being planted in pots, one hour after soaking in nodule infusion. They were also tested after 6 hours, 24 hours, 72 hours, 10 days, 30 days, 90 days, 6 months and 9 months.

The bacteriological technique used was as follows: 5 seeds (5 seeds weigh about 1 gm.) were taken from the bag with sterile forceps, placed in Erlenmeyer flasks containing 100 cc. of sterile tap water, allowed to stand 20 minutes, so as to thoroughly soften the seed to insure adhering cells being easily washed off, agitated 5 minutes in an electric soil shaker, and plated on modified Ashby's agar. The dilutions commonly used were: 1:1,000; 1:100,000; and 1:1,000,000. Plates were always poured in duplicate, hence 6 plates were required for each test. These were incubated at 25° C. for 10 days, after which they were counted.

For the pot tests, where seeds were tested for their ability to produce nodules, small earthenware pots, containing 2 pounds of soil were used. The pots were sterilized in an autoclave, at 10 pounds pressure, for 20 minutes. The soil used was a Sassafras sandy loam, taken from a remote part of the college farm, which had been shown repeatedly not to contain *B. radicicola* of the soybean or alfalfa variety. The soil was made up to optimum moisture content. Seven seeds were planted in each pot, 5 being allowed to grow for the period of the experiment. Duplicate pots were always planted to help

avoid possible errors. Distilled water was used at first to moisten the soil, but it was later found that the greenhouse tap water contained no *B. radicicola*, hence tap water was afterwards used. After 30 days the pots were removed to another building, the soil and plants removed, and the nodules carefully counted. The results of the bacteriological tests and the vegetation experiments obtained in the preliminary experiment are given in table 1.

TABLE 1

Results of preliminary experiments with Early Brown soybeans

LENGTH OF TIME AFTER INOCULATION THAT TEST WAS MADE		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT				
		2 minutes	20 minutes	1 hour	6 hours	24 hours
1 hour..	Bacteria per seed*	1,300,000	1,600,000	5,400,000	6,300,000	10,000,000
	Nodules per plant†	5.8	7.1	7.2	3.9	14.7
6 hours...	Bacteria per seed	720,000	1,300,000	4,200,000	900,000	7,300,000
	Nodules per plant	6.1	7.0	8.1	9.9	14.0
24 hours...	Bacteria per seed	360,000	620,000	3,100,000	3,900,000	5,300,000
	Nodules per plant	11.0	3.1	6.7	4.9	7.9
72 hours...	Bacteria per seed	390,000	520,000	750,000	1,100,000	3,900,000
	Nodules per plant	7.8	6.2	6.0	3.0	4.9
10 days...	Bacteria per seed	100,000	175,000	90,000	330,000	1,700,000
	Nodules per plant	3.9	2.8	3.3	7.1	3.7
30 days...	Bacteria per seed	Less than 100	15,000	100,000	210,000	900,000
	Nodules per plant	4.1	5.1	1.9	3.6	5.7
90 days...	Bacteria per seed	10,500	1,700	Less than 100	2,750,000	2,000,000
	Nodules per plant	0.3	0.4	0.1	1.0	0.2
6 months.	Bacteria per seed	Less than 100	Less than 100	Less than 100	3,100,000	1,800,000
	Nodules per plant	3.5	8.0	1.5	8.0	11.0
9 months.	Bacteria per seed	Less than 10	Less than 100	Less than 100	1,500,000	1,400,000
	Nodules per plant	0.8	4.5	5.0	6.0	4.0

* Modified Ashby's agar 10 days at 25°C.

† 30 days growth in greenhouse.

Table 1 shows that the Early Brown variety of soybean, when placed in an infusion of nodules for 2 minutes and subsequently dried and stored, retained viable organisms on the seedcoat for over 3 months. Those soaked 20 minutes and 1 hour retained the organism 6 months, and those kept in the infusion

as long as 6 to 24 hours had many viable nitrogen-fixing bacteria on their seedcoats after 9 months. In general the bacteriological results were in agreement with the vegetation experiments carried on in the greenhouse for the purpose of observing nodule production. It was found in general that the greater the number of organisms found on the seeds, the greater was the number of nodules per plant in the pot experiments.

There were several notable exceptions to this generalization, however. Several pots were planted and treated in exactly the same manner as the test pots, with the exception that they were well inoculated after planting with a rich nodule infusion. It was thought that this might give an indication of the efficiency of nodule production by the bacteria on the seeds being tested. The pots inoculated in this way gave, on an average, about thirty per cent more nodules than test plants. The data are not conclusive, hence no deductions are drawn. It is probable, however, that more nodules were produced for the reason that more nodule-producing bacteria were present. There were some discrepancies in the results, especially in the pot experiments, for example—Early Brown soybean treated 20 minutes and stored for 3 months gave an average of but 0.4 nodule per plant. After 6 months there was an average of 8 nodules per plant. In another case beans treated 6 hours produced but 1 nodule per plant after 3 months, while after 6 months it produced 8. What caused such fluctuations is not known, but that they are often met with in microbiological work is not new to research workers.

On the whole, then, the preliminary experiment shows that soybeans may be inoculated, if ordinary precautions against contamination are used and stored for periods of 3 months, without seriously affecting the viability of the organisms upon the seedcoats. It is not recommended that seeds be treated with an inoculant for periods over one hour, as the germinating power may be seriously injured. In view of these interesting results a more thorough investigation was planned and carried out as here described.

MAIN EXPERIMENT

Both soybean and alfalfa seed were used in this experiment. The variety of soybean used was the Black Eyebrow, while the common variety of alfalfa, grown in Arizona, was used. The portion of the tests dealing with soybeans will be taken up first, the plan of the experiment being briefly as follows:

1. Soybean seeds were treated with a nodule infusion for varying lengths of time, then removed, dried, stored and tested at fixed periods for the presence of *B. radicicola*. The test consisted of two parts, one bacteriological and the other vegetation, that is the seeds were planted in pots in the greenhouse and nodule formation observed.

2. This experiment was the same as no. 1, except that the seeds were treated after inoculation with a sterile 10 per cent gum tragacanth jelly.

3. Soybean seeds were treated for a varying length of time with a commercial culture of *B. radicicola*. The seeds were then dried, stored and

tested at fixed periods of time for the presence of the nitrogen-gathering organisms.

4a. Moist soybean seeds were inoculated with air dry infected soil, and tested after fixed periods for *B. radicicola.*

b. Soybean seeds were inoculated with a nodule suspension for 10 minutes and subsequently dusted with air-dry sterile soil. The purpose of experiment 4a and 4b was to determine whether soil either moist or air-dried would not aid in lengthening the viability of the organisms upon the seedcoats.

TABLE 2

Results of bacteriological and vegetation experiments with soybean seed inoculated with soybean nodule infusion

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	6 hours
1 hour.....	Bacteria per seed	245,000	4,000,000	2,000,000	2,300,000
	Nodules per plant	1.0	4.3	1.0	1.3
6 hours.....	Bacteria per seed	600,000	2,000,000	1,300,000	2,000
	Nodules per plant	1.0	1.3	4.0	1.3
24 hours.....	Bacteria per seed	8,000	250,000	580,000	94,000
	Nodules per plant	0.8	1.0	1.0	0.8
6 days.....	Bacteria per seed	55,000	85,000	205,000	50,000
	Nodules per plant	6.3	1.5	2.8	1.7
15 days.....	Bacteria per seed	20,000	80,000	90,000	18,000
	Nodules per plant	7.0	6.2	1.5	0
30 days.....	Bacteria per seed	4,000	95,000	450	20,000
	Nodules per plant	1.3	11.0	3.0	3.3
90 days.....	Bacteria per seed	Less than 100	600	330,000	1,850
	Nodules per plant	3.5	7.0	8.0	5.3
6 months.....	Bacteria per seed	2,000	9,500	2,700	1,100
	Nodules per plant	4.0	6.0	No plants	7.0

PROCEDURE

Procedure for the bacteriological test was the same as that used in the preliminary experiment. Mercuric bichloride, 1 to 500, for 5 minutes was used to sterilize the seeds, 5 of which were used in each bacteriological test made. The seeds were dried on sterile paper towels, in a quiet, dark room and afterwards stored in the dark, in sterile paper bags. Modified Ashby's medium was used throughout the experiment. The dilutions most commonly used were 1: 1,000, 1: 10,000 and 1: 100,000, although occasionally plates were

poured at higher or lower dilutions, especially if it was found that the number of cells present was extremely high or low.

The methods of testing nodule production in pots were practically the same as those already described in the preliminary experiments. A coarse red sandy soil, of very low fertility, was used in the pots. This sand came from a pit and was found negative several times for all types of *B. radicicola*. In each pot were placed 2 pounds of sand, to which had been added 1 gm. of

TABLE 3

Results of bacteriological and vegetation experiments with soybean seed treated with gum tragacanth and inoculated with nodule infusion

Experiment 2a

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH CULTURE			
		5 minutes	30 minutes	1 hour	6 hours
1 hour	Bacteria per seed	1,180,000	2,460,000	2,900,000	3,250,000
	Nodules per plant	0.5	1.3	2.2	1.5
6 hours	Bacteria per seed	850,000	450,000	2,850,000	105,000
	Nodules per plant	6.0	2.0	0.7	1.1
24 hours	Bacteria per seed	610,000	100,000	600,000	550,000
	Nodules per plant	1.3	1.0	2.4	5.1
6 days	Bacteria per seed	185,000	260,000	220,000	2,000
	Nodules per plant	4.4	6.2	1.4	2.1
15 days	Bacteria per seed	2,000	300,000	90,000	10,000
	Nodules per plant	11.0	2.6	1.0	1.0
1 month	Bacteria per seed	28,000	195,000	50,000	32,000
	Nodules per plant	14.0	2.9	2.5	3.7
3 months	Bacteria per seed	1,500	50,000	40,000	3,000
	Nodules per plant	3.2	5.2	1.0	13.0
6 months	Bacteria per seed	7,000	30,000	2,000	Less than 100
	Nodules per plant	8.1	4.6	7.6	7.2

potassium chloride, 1 gm. of acid phosphate and 2 gm. of calcium carbonate. All pots were sterilized in an autoclave at 10 pounds pressure for 20 minutes. Nodule counts were made after 30 days, but in some of the later experiments, this time was allowed to lapse a few days. It is not believed that this affected the results seriously. The results of the soybean experiments are given in tables 2 to 5.

The soybean nodule infusion used to inoculate the seeds contained 4,000,000,000 cells per cubic centimeter. It was made up by crushing fresh, well-

cleaned nodules in sterile water and filtering the liquid through 4 thickness of cheesecloth. The alfalfa-nodule infusion was prepared in the same way, but owing to the difficulty of obtaining nodules, the cells per cubic centimeter, numbered but 27,000,000. As a result of several tests both the soybean and alfalfa seeds used in the work were shown not to harbor any member of the *B. radicicola* group. Check pots planted from time to time also showed the soil used, to be negative for this organism.

TABLE 4

Results of bacteriological and vegetation experiments with soybeans and alfalfa seed inoculated with commercial cultures

Experiment 3

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH CULTURE			
		Soybeans		Alfalfa	
		5 minutes	1 hour	5 minutes	1 hour
6 hours	Bacteria per seed	100,000	3,000,000	812,000*	2,220,000
	Nodules per plant	2.8	19.0	12.5	12.0
72 hours	Bacteria per seed	490,000	1,900,000	170,000	280,000
	Nodules per plant	2.4	6.5	6.4	9.5
15 days	Bacteria per seed	60,000	530,000	360,000	800,000
	Nodules per plant	0	2.1	3.4	4.0
30 days	Bacteria per seed	27,000	70,000	650,000	50,000
	Nodules per plant	2.8	6.9	4.7	2.6
3 months	Bacteria per seed	3,000	58,000	430,000	24,000
	Nodules per plant	11.6	3.0	6.0	4.5
6 months	Bacteria per seed	500	5,000	18,000	2,000
	Nodules per plant	8.0	4.9	4.4	7.7

NOTE—The original soybean culture contained 91,000,000 cells per cubic centimeter while the alfalfa culture contained 600,000,000.

* Bacteria per gram of alfalfa seed.

DISCUSSION OF RESULTS

The data incorporated in tables 2 and 3 show that soybean seeds kept in contact with the inoculant for periods of time varying from 5 minutes to 6 hours, retain viable organisms on their seedcoats for at least 6 months. The greatest decrease in the number of organisms per seed took place during the first 6 days. In fact, the cells began to perish from the first hour. Those cells of low viability and those most exposed were undoubtedly the ones that died first, while the more resistant ones and those better protected, remained alive longest. There is but little correlation between the number of bacteria

per seed and the nodules produced per plant. From the data it appears that "physiological efficiency" (a term first suggested by Dr. J. G. Lipman) of the bacteria plays an important rôle in nodule production. It seems that those organisms which are most resistant to drying are those which are able to penetrate the root hairs of the plants and set up a symbiosis. It is probable that a great many living cells do not multiply on agar plates, hence this may account for the production of nodules from seeds showing very few cells. It

TABLE 5

Results of bacteriological and vegetation experiments with soybean and alfalfa seeds treated with air-dry infected soil

Experiment 4

STORAGE PERIOD		METHOD OF TREATMENT			
		Soybeans		Alfalfa	
		Moist inoculated seeds dusted with air-dry soil	Moist sterile seeds dusted with infected soil	Moist inoculated seeds dusted with air-dry soil	Moist sterile seeds dusted with infected soil
6 hours	Bacteria per seed	720,000	266,000	Lost	Lost
	Nodules per plant	7.7	8.2	8.5	5.8
72 hours	Bacteria per seed	Less than 100	100,000	180,000*	1,000,000
	Nodules per plant	2.8	4.5	1.7	4.2
15 days	Bacteria per seed	50,000	168,000	2,000	250,000
	Nodules per plant	1.1	1.0	4.1	3.9
30 days	Bacteria per seed	10,000	20,500	13,000	35,000
	Nodules per plant	0	6.0	0.8	3.4
3 months	Bacteria per seed	Less than 100	230	8,000	66,000
	Nodules per plant	9.0	4.9	5.0	1.9
6 months	Bacteria per seed	Less than 100	50	3,500	2,000
	Nodules per plant	4.2	5.4	3.0	0.9

NOTE—The soybean soil contained 14,000,000 *B. radicicola* per gram, while the alfalfa soil contained 6,600,000 cells per gram.

* Bacteria per gram of alfalfa seed.

was thought that this experiment might thrown some light upon the relation of the bacteria developing on agar plates and those producing nodules on plants.

It is certain that only a very few cells have the power to infect root hairs. Oftentimes seeds showing over a million bacteria when planted and grown under suitable conditions produced but one or two nodules per plant. The ratio, then, of the number of bacteria capable of developing on agar plates

to those capable of producing nodules is an exceedingly narrow one. Further study of this problem is sorely needed, especially studies which might show the factors influencing infectibility of the cells. It is impossible to explain in the light of our present knowledge of the subject, certain of the data. For example, in table 1, soybean seeds which have been soaked for 6 hours in the inoculant and which showed 3,250,000 bacteria per seed, gave but 1.3 nodules per plant, while similar seeds 6 months later showed less than 100

TABLE 6

Results of bacteriological and vegetation experiments with alfalfa seed inoculated with alfalfa nodule infusion

Experiment 1b

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	6 hours
1 hour.....	Bacteria per gram of seed	250,000	160,000	900,000	570,000,000
	Nodules per plant	4.7	3.1	7.0	2.1
24 hours.....	Bacteria per gram of seed	2,400,000	113,000	3,000,000	440,000,000
	Nodules per plant	7.6	3.3	3.2	3.5
6 days.....	Bacteria per gram of seed	3,750	5,800	2,070,000	8,000,000
	Nodules per plant	3.2	2.8	3.3	3.4
15 days.....	Bacteria per gram of seed	12,000	70,000	156,000	36,000,000
	Nodules per plant	0.4	1.7	0.3	1.1
1 month....	Bacteria per gram of seed	3,000,000	14,000	7,500	28,000,000
	Nodules per plant	3.2	3.1	2.3	2.3
3 months...	Bacteria per gram of seed	2,500	600*	4,000	2,500,000
	Nodules per plant	4.3	7.9	9.0	5.4
6 months...	Bacteria per gram of seed	53,000	5,000*	2,000	2,000,000
	Nodules per plant	4.0	0.8	4.1	4.9

* Poor plates.

cells per seed and produced an average of 7.2 nodules per plant! If there were any disturbing factors in the experimental work, the writer is not aware of them, as all ordinary precautions against contamination were taken.

In the case of seeds coated with gum tragacanth, the results are similar to those already recorded. The gum-coated seeds did not give sufficient protection to the bacteria to cause increased nodule production on the plants. The addition of gum, glue or even milk has been advocated by various persons to increase the longevity of the nitrogen-producing bacteria on legume seeds. This experiment indicates that the use of such substances is of doubtful value. As in the first experiment in every case bacteria survived on the seeds

for at least 6 months and were able to produce nodules on the roots of plants grown from these seeds. In this experiment also the number of bacteria per seed and the number of nodules produced per plant did not go together in general.

In table 4 are recorded the results of the inoculation of soybean and alfalfa seed with commercial cultures. The commercial culture used was Mulford's Nitrogerm in both cases. The directions were followed, the seeds being allowed to stand in the culture, for 5 minutes in one case and 1 hour in the

TABLE 7

Results of bacteriological and vegetation experiments with alfalfa seeds treated with gum tragacanth and inoculated with nodule infusion

Experiment 2b

STORAGE PERIOD		LENGTH OF TIME IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	8 hours
6 hours.....	Bacteria per gram of seed	350,000	210,000	170,000	Lost
	Nodules per plant	9.0	6.0	12.5	4.3
24 hours.....	Bacteria per gram of seed	335,000	2,220,000	10,000,000	33,000,000
	Nodules per plant	6.6	5.8	5.5	4.8
6 days.....	Bacteria per gram of seed	260,000	1,720,000	760,000	14,400,000
	Nodules per plant	2.9	3.4	2.3	3.1
15 days.....	Bacteria per gram of seed	120,000	120,000	Lost	8,000,000
	Nodules per plant	1.5	2.0	4.2	3.8
1 month....	Bacteria per gram of seed	720,000	130,000	2,000,000	7,200,000
	Nodules per plant	5.4	1.8	4.1	4.1
3 months...	Bacteria per gram of seed	1,500	36,000	520,000	3,000,000
	Nodules per plant	6.0	11.8	11.3	8.6
6 months...	Bacteria per gram of seed	No test	300,000	150,000	No test
	Nodules per plant	5.9	3.3	4.4	4.0

second case. Here the counts are much more uniform than those recorded in the previous experiments. The rate of dying out of the cells was much more uniform. This rate was most rapid in the first few hours, but after this it became more gradual, until at the end of 6 months the average number of bacteria per seed for both soybean and alfalfa was 6370, while the average number of nodules produced per plant was 5.5. These data show that *B. radicicola* withstands drying fairly well. This is not in accord with the experience of several other investigators. In this work, however, the initial inoculation was very rich and although the number of bacteria surviving for long periods of time was not great, yet those which did survive seemed to

retain their nodule-producing powers to a large extent. In case commercial cultures are used to inoculate seed it should be made certain that these cultures are rich in viable bacteria to insure living organisms remaining upon the seed-coats for a long time.

Referring to table 5, where data regarding seed inoculation by the use of soil are recorded, it is seen at a glance that under the conditions of the experiment soil did not aid in preserving the life of the bacteria. However, as in the other tests, viable cells persisted on the seed for over 6 months and, moreover, were able to produce nodules on the young seedlings. At the end of 30 days there was still an average of about 15,000 viable cells per soybean seed and about 25,000 cells per gram of alfalfa seed. The results are similar in the case of both of these legumes. Of the two inoculation methods tested out, it appears to be much more efficient to treat the moist uninoculated seed with air-dry infected soil than to treat the moist inoculated seed with sterile air-dry soil. The soil used to inoculate the soybeans contained 14,000,000 bacteria per gram, while the alfalfa soil contained 6,600,000 per gram. The soybean soil, when plated after 30 days, showed 70,000 *B. radicicola* per gram, while the alfafa soil showed none. The soil used in this experiment was a fine Penn loam, which had been sterilized by heat previous to inoculation with *B. radicicola*. In the tests, in which alfalfa seed was used, the experimental procedure was the same as with the soybeans. For the bacteriological tests, glass tubes containing 1 gm. of seed were used in place of individual seeds as was done in the soybean tests. This was done because of the small size of the alfalfa seed and also to reduce the experimental error.

Turning to tables 7 and 8, one sees that the results obtained by drying the alfalfa organism, under various conditions, are in accord with those already found to be true with the soybean bacteria. There does not seem to be much difference in the resistance of these two varieties of *B. radicicola* as regards desiccation. Although more bacteria are present on seeds soaked for a long time in the infusion, yet in general, the shortest period of time the seeds were kept in contact with the inoculant (i.e. 5 minutes), gave nearly as good results as longer periods of time. This was also found to be true by and large in the case of soybeans inoculated with either nodule infusion or commercial cultures. It is recommended, therefore, that 10 minutes is sufficient time to allow seeds to remain in contact with the inoculant. Seeds soaked over one-half hour and subsequently dried have their germinating power somewhat impaired. This was shown to be the case, when both soybean and alfalfa seeds were kept in contact with the liquid for over half an hour. The germination of the soybeans was injured more than that of the alfalfa.

In the case of alfalfa seed treated with gum tragacanth, the results obtained do not justify the use of such substances. It probably aids to some extent in protecting the bacteria, but not enough to justify the cost and trouble of using it. The data contained in these two tables show that the nodule organism is capable of withstanding considerable desiccation, and that the

inoculated seeds were able to produce plants having abundant nodules, when planted at fixed periods up to and including 6 months.

During the progress of the experiment, it was decided to reinoculate with a rich nodule infusion some of each series of pots. Theoretically, at least, this should give important data regarding the value of the two methods of inoculation, as well as whether the addition of a great number of bacteria would produce more nodules per plant than fewer cells. The data, as compiled in table 8, show the proportion of nodules produced in pots where the number produced as a result of inoculated seed alone, is taken as unity, compared with the nodules produced by both inoculated seeds and inoculated soil. Both soybeans and alfalfa were used.

In the soybean tests it is seen that the proportion of nodules produced from the inoculated seeds alone to the soil plus seed method was fairly constant. The average figure is 1:2.9. That is, the addition of inoculating material to

TABLE 8

Proportion of nodules produced in pots where inoculation by seed alone is taken as unity and compared with inoculation by both seed and soil

LENGTH OF STORAGE OF INOCULATED SEEDS BEFORE PLANTING	NUMBER OF POTS IN TEST		PROPORTION OF NODULES PRODUCED BY INOCULATED SEED ALONE TO INOCULATED SEED + INOCULATED SOIL	
	Soybean	Alfalfa	Soybean	Alfalfa
1–24 hours	6	20	1:2.4	1:1.2
48 hours	1	3	1:1.8	1:1.3
6 days	4	12	1:1.7	1:1.0
15 days	4	8	1:2.1	1:1.5
3 months	5	5	1:3.7	1:0.8
6 months	3	2	1:1.8	1:3.9

soil produced more nodules per plant than where inoculated seeds alone were used. The length of storage did not appear to make a very great difference in this proportion.

In the case of alfalfa, the proportion is narrower, i.e. 1:1.7. Here also the addition of inoculating material to the pot caused a proportionate increase in the number of nodules. Therefore, with both alfalfa and soybean seeds, the number of nodules per plant is increased by inoculating the pots with a nodule infusion in addition to planting inoculated seeds. These data are somewhat contradictory to those obtained in the previously reported experiments, where it appeared that numbers of bacteria did not seem to influence the nodule production greatly.

SUMMARY

The following statements seem justified as a result of the experiments here described.

1. Seeds may be satisfactorily sterilized by the use of mercuric chloride, 1:500 kept in contact with the seed for 5 minutes.

2. The methods herein described for carrying on bacteriological and vegetation experiments for testing the viability of *B. radicicola* on seeds, give very good results.

3. Soybean or alfalfa seeds inoculated with a nodule infusion retain viable organisms on their seedcoats 6 to 9 months. Plants grown from these seeds had nodules on their roots, showing that the bacteria had not lost the infecting power.

4. The soybean and alfalfa nodule organisms seem to be able to withstand nearly the same degree of desiccation.

5. The shortest period of time, i.e. 5 minutes, that the seeds were kept in contact with the inoculant gave nearly as good results as longer periods of contact.

6. Gum tragacanth and probably similar substances do not aid greatly in keeping alive the bacteria upon the seeds. Their use is not recommended.

7. Infected dry soil, dusted upon moist seeds, gave better results than sterile, dry soil dusted upon moist inoculated seeds. The seeds inoculated with soil did not retain *B. radicicola* longer on their coats, neither did the plants have a greater number of nodules, than where nodule infusion or commerical cultures were used.

8. Commercial cultures, when used to inoculate soybeans or alfalfa, give as good results as nodule infusion.

9. In general the number of bacteria per seed and the number of nodules per plant did not correlate closely.

10. It appears that but an exceedingly small percentage of the cells present on a seed are able to produce nodules on the roots of the seedling. This ratio may be as narrow as 1: 1,000,000.

11. It seems probable that those cells which resist desiccation longest are those which have the greatest infectibility.

12. The period which showed the greatest destruction of cells was the first few hours after inoculation; after this the cells perished relatively more slowly, and uniformly.

13. The inoculation of soil in pots by means of a rich nodule infusion increases considerably the number of nodules produced per plant over the number produced by inoculated seed alone.

14. It is not recommended that inoculated seeds be stored for long periods of time before planting, but ordinarily a delay of several days, or even a month should do no great harm. There seems to be little difference in the resistance to desiccation whether nodule infusion, commerical cultures or soil be used as the infecting agent.

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SOME NOTES ON THE CAUSE OF THE UNPRODUCTIVITY OF "RAW" SUBSOILS IN HUMID REGIONS

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In a recent paper, Harmer (3) has reported vegetation experiments showing that some Minnesota subsoils are as productive towards alfalfa when inoculated as are the corresponding surface soils, while others are much less productive and hence may properly be considered "raw." His experiment involved no attempt to determine the cause of the unproductivity where it existed, and, as he was unable to continue the study, the writer has used the remaining portions of the bulk sample which he had collected at two of the places, viz. Canby and Renville.

The possibility of a deficiency of available potash or phosphoric acid or both as the cause of the "rawness" in such cases has been discussed by Lipman (4, p. 290), Alway (1) and Alway, McDole and Rost (2, p. 10).

The object of the experiment described below was to determine whether the addition of potash and phosphoric acid would fully eliminate the unproductivity.

EXPERIMENTAL

The origin and method of collecting the bulk samples and their properties have been fully dealt with by Harmer, and it will suffice here to state that Canby and Renville are in the prairie region of west central Minnesota and that the soils were taken from pits in virgin prairies and shipped to the experiment station in the autumn of 1916. The portions used in the experiment had remained in sacks until the fall of 1917. The properties of the soils are reported in table 1 (3, p. 395).

Owing to the limited quantity of the soils remaining, it was not possible to carry out the pot experiments in duplicate, but as Harmer (3, p. 400) has shown, the use of single pots may be expected to lead to the same conclusions as do duplicates.

The 4 air-dried soils, both surface and subsoil, were prepared by mixing and passing through a coarse screen. Half of each was placed directly in a vegetation vessel, while the other half was first treated with disodium phosphate and potassium sulfate, each in amounts equivalent to 1000 pounds per acre. The soils were spread out upon large sheets of oilcloth, the powdered

salts sifted over them and the whole thoroughly mixed and transferred to the vegetation vessels. These were of galvanized iron 12 inches deep with a surface area of 0.75 square feet and painted on the inside with pitch tar, drainage being effected by holes in the bottom and a layer of coarse gravel. The treatments are shown in table 2. The soils were brought to approximately the moisture equivalent and the pots allowed to stand 48 hours, and, on December 3, 1917, planted to alfalfa. When the plants were up and several inches in height they were thinned to leave 30 plants in each pot. The inoculation had already been assured.

In all, 5 crops were harvested, the first on April 25, the last on November 20. The plants on the untreated subsoils were spindling and without much foilage, although their color was as good as any. In table 2 the yields are reported. The increases due to the treatment are shown, it being assumed for the purpose that the yield on each of the unfertilized soils was 100. It will be seen that in the case of the two surface soils which received no treat-

TABLE 1

Composition and properties of the soils used

LOCATION	DEPTH	MOISTURE EQUIVALENT	NITROGEN	ORGANIC CARBON	ORGANIC MATTER*	REACTION OF THE SOIL
			per cent	*per cent*	*per cent*	
Canby	1 to 6 in.	30.25	0.329	4.015	6.922	Neutral
	Third foot	23.24	0.051	0.770	1.328	Neutral
Renville	1 to 6 in.	35.91	0.393	5.134	8.852	Neutral
	Third foot	27.28	0.051	0.865	1.491	Neutral

* Computed from organic carbon. Organic matter = Organic carbon × 1.724.

ment the yields were very similar, amounting to totals of 60.5 gm. for that from Canby and 62.4 gm. for that from Renville, equivalent to almost 4 tons of hay per acre. The applications of potash and phosphoric acid caused appreciable increases in the yields on these surface soils, 28 per cent in the case of that from Renville and 39.1 per cent with that from Canby. On the unfertilized subsoils the yields were very low, only one-fifth to one-third as high as on the corresponding surface soils. In the case of the fertilized subsoils, however, there was a very great improvement in growth, the appearance of the plants was exceptionally good and much higher yields were obtained at each harvest, even better than on the unfertilized surface soils, the total for the 5 crops being equivalent to over 4 tons of hay per acre compared with 0.85 tons on the unfertilized. The lowest increase noted was in the case of the Canby subsoil at the time of the cutting of the fourth crop, viz. 160.6 per cent, while the highest was with the first crop on the Renville soil which gave over 1150.0 per cent increase. For the 5 crops the increase for the Renville subsoil was 472.1 per cent and for the Canby subsoil 309.3 per cent. From these data it is to be seen that by the additions of readily available potash

and phosphoric acid the "rawness" of the subsoils, as evidenced by the growth and yield, had been eliminated, the alfalfa making as vigorous a growth and giving as high a yield as on the corresponding surface soils which had received no fertilizer.

TABLE 2

Yields of dry matter from 5 crops of alfalfa

SOIL	TREATMENT	FIRST CROP APRIL 25	SECOND CROP MAY 24	THIRD CROP JUNE 26	FOURTH CROP AUGUST 1	FIFTH CROP NOVEMBER 20	TOTAL FOR 5 CROPS	TOTAL FOR 4 CROPS IN EARLIER EXPERIMENT*
				Renville				
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Surface 6 in.	Nothing	4.0	12.9	21.1	14.3	10.1	62.4	70.3
Surface 6 in.	Potash and phosphoric acid	5.9	14.0	24.9	18.8	15.3	79.9	
	Increase (per cent)	47.5	8.5	18.0	31.4	51.5	28.0	
Third foot..	Nothing	0.2	2.9	3.7	3.4	1.3	11.5	15.3
Third foot..	Potash and phosphoric acid	2.5	16.7	18.1	16.6	11.9	65.8	
	Increase (per cent)	1150.0	475.5	389.2	388.2	815.3	472.1	
				Canby				
Surface 6 inches..	Nothing	4.5	13.5	17.5	14.6	10.4	60.5	51.8
Surface 6 inches..	Potash and phosphoric acid	3.2	20.3	23.8	19.6	17.3	84.2	
	Increase (per cent)		50.3	36.0	34.2	66.3	39.1	
Third foot..	Nothing	0.2	3.1	5.2	6.1	2.6	17.2	19.8
Third foot..	Potash and phosphoric acid	1.6	14.9	24.7	15.9	13.3	70.4	
	Increase (per cent)	700.0	380.6	375.0	160.6	411.5	309.3	

* Harmer (3, p. 398).

The above described experiment proves that the "rawness" shown to exist in some subsoils may be removed by an application of potash and phosphoric acid, and hence in them it is probably due to a deficiency of available mineral nutrients.

SUMMARY

Vegetation experiments with certain Minnesota prairie subsoils previously found "raw" towards inoculated legumes have shown that an application of soluble potash and phosphoric acid fertilizers removes this infertility, rendering the subsoils as productive as the corresponding surface soils, evidence that "rawness" in these cases is due to a lack of readily available mineral nutrients.

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THE EFFECT OF SOYBEAN GERMINATION UPON THE GROWTH OF ITS NODULE-FORMING BACTERIA[1]

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The study of the influence of plants upon soils is becoming quite as important as that of soils upon plants (14). It is well known that certain crops require the presence of a particular kind of bacteria in the soil in order to yield the largest return. When the proper nodule-forming bacteria are entirely absent from the soil or are present in relatively small numbers, it is customary to inoculate, i.e., to add the bacteria. Inoculation may be carried out by one of two ways: by the transfer of soil containing the particular kind of bacteria or by the less laborious method of sowing inoculated seed.

It has been shown that plants exert an appreciable influence upon the number of bacteria in soil (11). The question may be raised as to whether or not the first stages of the plant's active growth or, to be more specific, whether or not the germination of legume seed, has an injurious effect upon the growth of the nodule-forming bacteria with which the seed have been inoculated.

In an extensive series of investigations, Hiltner and Störmer (10) have pointed out that direct inoculation of large seeds such as lupine and soybean, may not lead to nodule formation and have concluded that the failure to secure inoculation was due to the excretion of substances from the seedcoats during the process of swelling. In their investigations, soybean seed were covered with distilled water for 5 hours and the water extract was used in studying the growth of the bacteria and their transformation into bacteroids.

As a result of their studies upon nodule bacteria in aqueous extracts of seedcoats and of the contents of legume seed, they conclude that a protein is given off from the seed contents that favors the growth of the nodule bacteria, whereas from the seedcoats substances are given off that may help to plasmolyze and destroy the bacteria. They suggest the possibility of antagonism between the substances given off by the seed contents and by the seedcoats that reduces the toxic action of the seedcoat excretion upon the bacteria. It seemed important, therefore, to investigate the toxicity of certain legume seed during their germination, for the nodule bacteria inoculated upon the seedcoat.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The method of experimentation consisted in adding a water suspension of the nodule bacteria to bacteria-free seed in a Petri dish, followed by sterilized Ashby's agar (1). The plates were rotated after the addition of the bacterial suspension, as well as after the addition of the agar. The former rotation insured the inoculation of the seed, whereas the latter rotation distributed the remainder of the bacteria uniformly throughout the plate. The uniform distribution of the bacteria made it possible to observe more readily any toxic effects upon the growth of the bacteria. The seeds were then distributed in the agar by means of a sterilized platinum needle.

The Ashby agar (7) contained sucrose in the place of mannitol, the dibasic instead of monobasic potassium phosphate, and no calcium carbonate. The seed used, unless otherwise specified, were medium yellow soybeans. The sobyean nodule bacteria were obtained from a growth on Ashby sucrose agar. Several loopfuls of the culture were added to a flask containing a small volume of sterilized distilled water and after thorough shaking, transfer of one cubic centimeter of the suspension to the plates was effected by means of sterile pipettes.

Sterilization of the seed was at first carried out by means of the autoclave with the consequent death of the cells. Wisconsin black, medium yellow, and medium early green soybean seed were sterilized in the autoclave in tubes and then sown in plates as described. If substances that are given off by the dead seed exert a toxic action upon the growth of the nodule bacteria, we should expect to obsetve an inhibition in the growth of the bacteria about the seed. In no case was an inhibition of the growth of the bacterial colonies apparent, although it was difficult to determine the relative number and size of the colonies near the seed, due to the diffusion of opaque substances from the dead seed. It is possible that heat sterilization destroyed the toxic substances if such were present in the living seed, although previous investigators (10) sterilized the water in which the soybean seed had been soaking.

The seedcoats of the three varieties of soybean seed were extracted with water and the extract sterilized in the autoclave. The extracts were added to sterile pieces of quantitative filter paper upon Ashby's sucrose agar containing a uniform inoculation of nodule bacteria. After a week's incubation of the plates at 27°C. there was no inhibition of growth of the colonies about or beneath the paper. The extract of the Wisconsin black soybean seed, after being autoclaved, still possessed the properties of an indicator of hydrogen-ion concentration.[2] The other two varieties did not contain an indicator in their seedcoats.

[2] It may be of interest at this point to describe the indicator that was found in the Wisconsin black soybean seed. The seedcoats were extracted with 50 per cent alcohol for 5 hours at 30°C. and gave a wine red solution. The same amount of indicator (5 drops) was placed in tubes containing equal quantities of buffer solutions of known P_H value. The color changes were mostly of the gradual transition type in going from P_H 1–10, being pink to red on the more acid side of the neutral point for distilled water and from pink-gray to

The seedcoats of the three varieties of soybean seed were removed, and the seedcoats and the seed contents of each variety sterilized in separate tubes in the autoclave. When placed on the usual medium, as described, and incubated for a week, no inhibition of the growth of the colonies of nodule bacteria was apparent.

It was desirable to sterilize the soybean seed in such a way as to eliminate the use of the autoclave which killed the cells. That sterilization (16) offered considerable difficulty is readily understood. Here it is essential not only to rid the seed of any adhering microorganisms, but also to take precautions not to replace such organisms by a sterilizing agent that is harmful to the bacteria that are to be studied. To this end it will be helpful to mention the observations upon the sterilization of yellow soybean seed by the use of a 0.25 per cent solution of mercuric chloride (15).

The sterilization apparatus was essentially that described by Fred (7). It consisted of a series of milk bottles connected by means of glass tubing with rubber connections and clamps. The apparatus was a closed system consisting of several empty bottles, a bottle of distilled water, and a bottle of mercuric chloride. It was then mounted for convenience on a board and sterilized in the autoclave. During the process of seed sterilization, a water suction pump was used to exhaust some of the air from the system. The seeds were covered with the mercuric chloride solution for seldom over 3 minutes (the time being regulated according to the type of seed used), and then were repeatedly washed under reduced pressure with sterilized distilled water, after which they were transferred to a sterile dish and were ready for use.

Sterile Petri dishes, containing the $HgCl_2$-sterilized yellow soybean seed, aqueous suspension of soybean bacteria, and Ashby's sucrose agar, were incubated for about a week (5 to 8 days) at 27°C. and the distribution and growth of the colonies observed in relation to the germinating seed. In order to show more clearly any injurious effects that the substances excreted from the seed might have upon the growth of the bacteria, the suspension of bacteria was heavily seeded with the nodule bacteria. The Petri dishes in some experiments were placed upright, while in others they were inverted; however, the position was found to have no effect on the results.

When the yellow soybean seeds were sterilized by the mercuric chloride method and were sown in the manner described, the condition shown in plate 1, figure 2, was observed. The plate had been inverted in order to permit the rootlets to grow in the moist chamber beneath the medium, and, for the purposes of photography, the germinated seeds were removed from the plates.

yellow-brown on the alkaline side. Between P_H 6 and 8, it was possible to detect color differences for each 0.2 change in P_H, the most marked changes being from about P_H 7–7.6. After the extract had been sterilized in the aotoclave and was left standing for about two weeks, the indicator lost many of its properties, in that the different buffer solutions gave only varying degrees of yellow or brown color.

It will be noted that a zone extends about each cavity in the medium where the seeds had been imbedded. The plates showed clear and transparent zones, whereas the remainder of the medium had somewhat of an opaque appearance. Under the microscope the clear zone was found to be entirely devoid of colonies. At the outer boundary of the clear zone the colonies were as numerous as anywhere else in the medium. The limiting line, up to which the colonies could still grow, was sharply defined and at this line there appeared to be a piling up of the colonies, possibly a region of stimulating concentration of the mercuric chloride. When medium early green and also Wisconsin black soybean seeds were used, similar results were obtained.

The question naturally suggested itself, are such zones due to the excretion of a substance by sterilized seed that is toxic to the growth of soybean nodule bacteria? In order to control the experiments, small squares of unhardened quantitative filter paper were sterilized with the soybean seed, using the mercuric chloride method and were subsequently treated as though the paper were actual soybean seed.

Plate 1, figure 1 shows the result of the experiments using such filter paper as described. The soybean seed, sterilized with the filter paper, gave results similar to those shown in plate 1, figure 2. While there were no such sharply demarcated clear zones about the pieces of filter paper, there were some areas near the filter paper that were unaffected, other areas that had a reduced number of colonies, and still other areas possessing no colonies whatsoever, but showing a more gradual transition to an increasing number of colonies farther away from the paper. The filter paper must have retained some (however small a quantity) of the mercuric chloride in the sterilization process, some of which it later gave up to the medium.

It was a simple matter, then, to ascertain whether or not the giving off of mercuric chloride by the seed or by the filter paper (that was sterilized with the seed) was responsible for the observed clearing of the medium. A sterile platinum loop bearing a thin film of mercuric chloride solution such as was used in the sterilization process, was touched against the medium at places marked upon the plates and after two days the plates were re-examined. Some of the plates that were used contained Ashby's sucrose agar alone, whereas others contained in addition very numerous nodule bacteria colonies. On both kinds of plates the touched areas were found to have cleared as in the clear zones of plate 1, figure 2. The clearing was due to the action of the mercuric chloride upon the Ashby sucrose agar. The presence of very numerous bacterial colonies about such zones tends to increase the contrast between the cleared and uncleared areas.

Evidently it is most difficult, if not impossible, in the sterilization of soybeans by the use of mercuric chloride solution with reduced pressure, entirely to remove the last trace of the mercuric chloride. The use (13) of mercuric chloride (3) as a means of sterilizing seed is a common practice. For many

purposes its use cannot be questioned, though it is not safe to assume that because the germination of the seed has not been injured, the mercuric chloride has been entirely removed.

Having found the use of mercuric chloride objectionable as a sterilizing agent (2, 12) for the soybean seed, other methods of sterilization were tried. Yellow soybean seeds were dipped (singly) a few seconds in concentrated sulfuric acid and immediately washed with large volumes of sterile distilled water under reduced pressure. This method is open to the objection that in dipping the seeds from the acid into the water, the temperature of the seedcoat cells is raised and perhaps the nature of the excreted substances changed. Some of the seeds were dipped in alcohol and rapidly flamed before being dropped into a sterile Petri dish. Other of the seeds were flamed directly. Such methods are open to the same criticism of undue heating and, even though germination was seldom affected, it was frequently difficult to secure seeds free of bacteria.

The writers found it desirable, in studying the effect of substances given off by germinating soybean seeds upon the growth of nodule bacteria, to remove all possible objections as regards the sterilization of the seed. Accordingly, yellow soybean pods, that were dry but intact, were secured. Some of the pods were carefully opened (8, 9) upon sterile dishes and the seed transferred to Petri dishes, after which the usual procedure of adding the bacteria and the medium was carried out.

As variants upon this method, some pods were dipped in alcohol, flamed, and the seed removed; other pods were opened and the seed dipped singly into alcohol and flamed. The seeds of some pods were given the sulfuric acid treatment, while the seeds of other pods were removed from the intact pods and transferred to the sterilizing apparatus (7) without the use of mercuric chloride or any other antiseptic agent, and were repeatedly and rapidly washed with large volumes of sterile distilled water under reduced pressure. The sterilization processes seldom required more than 3 to 5 minutes, the shortest possible time being used in every case in order not to cause any considerable leaching during the sterilization process.

When the plates secured from such seed had been incubated for 5 to 8 days at 27°C., it was found that in every instance, the nodule bacteria colonies were growing up to and about the germinating seed. In the neighborhood of the seeds there was a tendency for the colonies to increase both in size and in number, possibly due to the excretion of salts from the seed. In no case was there a clear zone or an inhibition to the growth of the colonies observed.

The sulfuric acid treatment or the flaming process did not give results that differed from the other methods of seed sterilization, but when carried on without the proper precautions there appeared visible effects upon the cotyledons. The seed that had been picked with sterile forceps from pods and had been repeatedly washed with sterile distilled water under reduced

pressure showed only an occasional contamination. When the same procedure was followed, except that the pods were momentarily dipped into alcohol and quickly drawn through the flame, no contamination was ever encountered.

Differences in susceptibility to inoculation between varieties of soybeans and even in some cases total resistance to inoculation, as had been reported by several investigators, has been shown by Leonard (13) not to exist in the 19 varieties of soybeans tested. The smoothness and oily nature of soybean seedcoats has been suggested (5) as possibly presenting some of the difficulties encountered in soybean seed inoculation. It might be mentioned in this connection (5) that frequently the difficulty in securing soybean nodules has been due to the inoculation of the seed with unreliable cultures. The distribution (4, 8) of tested cultures of nodule bacteria offers an effective control.

SUMMARY

It has been found that in studying the effect of soybean seed germination upon the growth of its nodule bacteria it is not permissible to use mercuric chloride as a sterilizing agent. In studies upon yellow soybean germination, it has been shown that the germination of the seed favors the growth of the bacteria. When bacteria-free soybean seeds were obtained directly from the pods and inoculated with soybean bacteria, no toxic action of substances excreted from the germinating seed upon the growth of the nodule bacteria was observed.

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PLATE 1

FIG. 1. Pieces of filter paper on plate.
FIG. 2. Effect of germinating soybean seeds on plate.

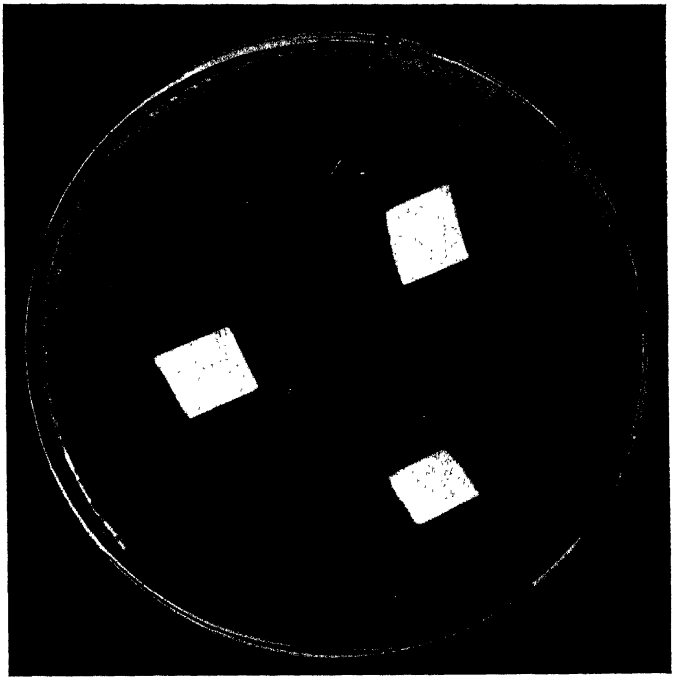

FIG. 1

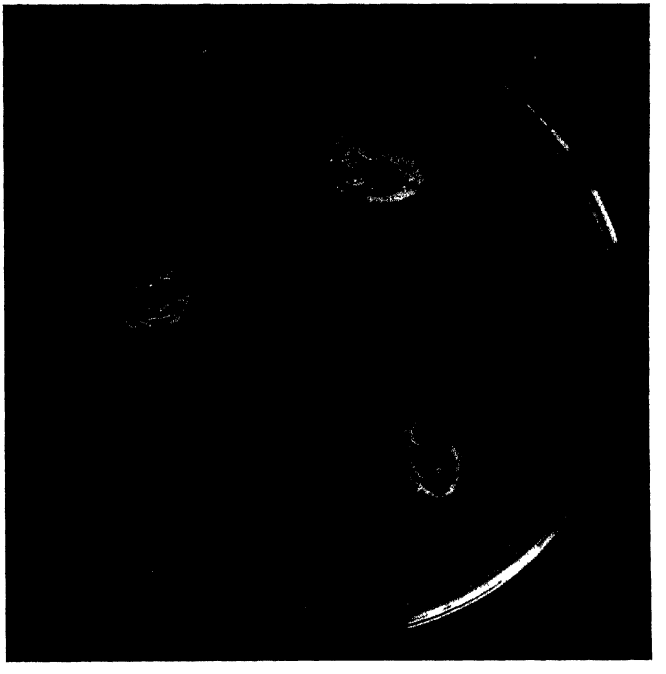

FIG. 2

THE ABNORMALITY OF SOILS IN FIELD-PLACED CYLINDER EXPERIMENTS

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In soil nitrogen studies (2) for the past 10 years at the Tennessee Agricultural Experiment Station, galvanized iron cylinders have been used to apparent advantage. The cylinder diameter was 2.225 feet, which gives a soil exposure of $\frac{1}{10,000}$ acre. Cylinders of both 4-foot and 1-foot depth were used and in both cases rested with open ends on undisturbed subsoil. The 4-foot cylinders were filled with subsoil and soil in layers as found in the field. For the 1-foot cylinders only surface soil was used. In these experiments no artificial watering was given, the idea at the outset being to allow the crops to grow under as nearly natural conditions as possible.

In the 4-foot cylinders—series A to G inclusive—two crops, wheat and millet, were grown each year for the first 5 years. For the following 3 years only cowpeas were grown. The 1-foot-cylinder experiments have run only 4 years. Japan clover was grown the first season and cowpeas each year for the following 3 years.

A striking feature of the results obtained from every one of the 5 soils used has been the rapid decrease in crop yield, resulting in the complete failure of the crops in the course of a few years except where lime was applied. At least three of these soils, Cookeville, Crossville and Farm, are known to be in immediate need of lime in order to produce either maximum crops of cowpeas or satisfactory crops of red clover. All three are loamy soils, but differ in color, texture, and fertility, only the last mentioned being considered as highly productive. The fourth, or "Gallatin" soil, is a brown loam of high natural fertility. The fifth, or "Jackson" soil, is a grey-colored silt loam, ordinarily considered of poor texture and structure, but well enough supplied with lime so that clover and even alfalfa were little benefited by liming in field experiments. However, as is usual for Tennessee soils, chemical analysis did not show the presence of an appreciable amount of carbonate of lime.

Although there were wide differences in natural fertility between the different soils, even the poorest, the Cookeville and Crossville, were not so poor but that harvestable crops would be expected for many years. The field production of the Farm soil has been under constant observation by the writer for the past 14 years and of the Jackson soil for the past 8 years, and

TABLE 1

Relationship between liming and continuity of crop production in 4-foot-cylinder experiments
(Soils placed in 1908; first crop harvested in 1909)

SOIL	CYLINDER NUMBER	TREATMENT*	YEAR OF FIRST CROP FAILURE	FIRST CROP TO FAIL	REMARKS—WEIGHTS OF AIR-DRY GRAIN AND STRAW
Cookeville...	A4	O	1912	Millet	1912 wheat crop 3.21 gm.
	A2	F	1914	Wheat	1913 millet crop 1.21 gm.
	A1	L			1914 wheat crop 39.03 gm.
	A3	LF			1914 wheat crop 62.69 gm.
	A7	PK	1912	Millet	1912 wheat crop 23.24 gm.
	A9	PKN	1912	Millet	1912 wheat crop 38.67 gm.
	A10	LPKN			1914 wheat crop 94.74 gm.
Crossville....	C4	O	1911	Wheat	1910 millet crop 14.15 gm.
	C2	F	1913	Wheat	1913 millet crop 1.04 gm.
	C1	L			1914 wheat crop 11.83 gm.
	C3	LF			1914 wheat crop 60.63 gm.
	C7	PK	1911	Wheat	1912 millet crop 0.36 gm.
	C9	PKN	1911	Wheat	1912 millet crop 28.97 gm.
	C10	LPKN			1914 wheat crop 50.84 gm.
Gallatin.....	E4	O	1913	Millet	1914 wheat crop 12.75 gm.
	E2	F	1914	Millet	Failure of millet in part due to season. 1914 wheat crop 163.10 gm. Cowpea crop failed in 1915 and thereafter.
	E1	L			1914 wheat crop 133.27 gm.
	E3	LF	1914	Millet	Failure of millet due to season. 1914 wheat crop 171.68 gm.
	E7	PK	1914	Millet	Practically no millet in 1913. 1914 wheat crop 4.77 gm. Cowpea crops failed in 1915, 1916 and 1917.
	E9	PKN	1914	Millet	1913 millet crop 0.63 gm. 1914 wheat crop 174.50 gm. Small cowpea crops harvested in all three years.
	E10	LPKN			1914 wheat crop 234.18 gm.
Jackson.....	G4	O	1917	Cowpeas	1914 wheat crop 74.21 gm.
	G2	F			1914 wheat crop 89.37 gm.
	G1	L			1914 wheat crop 96.41 gm.
	G3	LF			1914 wheat crop 89.07 gm.
	G7	PK			1914 wheat crop 65.28 gm.
	G9	PKN			1914 wheat crop 167.60 gm.
	G10	LPKN			1914 wheat crop 170.47 gm.

*The symbols of the third column are interpreted as follows:
F 6 tons of farmyard manure per acre applied in 1908 and again in 1911.
L 2 tons of ground limestone per acre applied in 1908.
P 300 pounds per acre of 16 per cent acid phosphate applied annually until 1915.
K 100 pounds per acre of muriate of potash applied annually until 1915.
N 160 pounds per acre of nitrate of soda applied annually until 1915.

both continue to be productive without the addition of either manure or lime, although the former responds readily to both materials and the latter to manure.

The accompanying tables give the essential data relating to these experiments. In the case of the 4-foot cylinders the results show that without lime the Crossville soil ceased to produce either wheat or millet after 2 years. Similarly, production ceased in 3 years for the Cookeville soil, and 4 years for the Gallatin soil, but continued for about 8 years for the Jackson soil. As a rule when production once ceased all future seedings resulted in failure. Particular attention is called to the fact that annual applications of soluble phosphate, potash and nitrogen did not extend the cropping period and that light applications of manure delayed crop failure only a couple of years for the Cookeville and Crossville soils.

TABLE 2

Yields of air-dry cowpea hay from Farm soil in 1-foot cylinders

(Soil placed in 1913. All manure and lime treatments made in 1913)

CYLINDER NUMBER	TREATMENT PER ACRE	HAY YIELDS		
		1915	1916	1917
		gm.	*gm.*	*gm.*
1	Burnt lime, 2 tons	107.5	246.5	216.0
4	Ground limestone, 4 tons	78.5	214.0	165.8
6	None	89.8	150.0	0.0
12	Manure, 12 tons	122.0	278.0	1.3
7	Burnt lime, 2 tons; manure, 12 tons	182.8	285.0	261.0
10	Ground limestone, 4 tons; manure, 12 tons	208.5	284.5	211.5
18	Manure, 30 tons	309.3	299.5	134.5
13	Burnt lime, 2 tons; manure, 30 tons	389.5	402.3	442.8
16	Ground limestone, 4 tons; manure, 30 tons	294.3	369.0	312.8
30	None	164.3	221.8	34.5

For the 1-foot-cylinder experiments, the yields of all the cowpea crops are given in table 2. The 1914 crop of Japan clover is omitted because the stands were very irregular so that there was no apparent relationship between the treatments and the crop production.

The results show that where no lime was applied the crops were practically failures at the end of 4 years. Even moderate to heavy applications of manure, as made at the outset, failed to do more than delay complete crop failure. On the other hand, the addition of either burnt lime or ground limestone enabled the production of excellent crops with no indication of immediate failure.

To account for the results, the writer sees only one unusual condition, the prevention of all run-off, that is, the projection of the cylinders for two or three inches above the surface of the ground forces the rainfall to stay in the cylinders so that an increased moisture supply, and at times excessive leaching, is the result. Apparently, therefore, the supply of bicarbonate of lime

was so greatly reduced under these conditions as to become the limiting factor and to bring about total crop failure in a very few years. Under field conditions, on the other hand, with less loss by leaching, crops continue to be produced, although for 4 of the 5 soils liming would be beneficial.

In view of these facts, the question may be raised as to the effect of the cylinder conditions on the availability of soil nitrogen, and, for that matter, on all the soil elements of plant-food, for soils of this character under similar climatic conditions.

In the absence of satisfactory comparisons of the losses of nitrogen under field and cylinder conditions, recourse can be had only to generalizations which are liable to more or less error. Perhaps they will serve to raise a question which should be settled by more definite evidence. The prevention of run-off —which has been estimated as high as 50 per cent of the total rainfall (1)— gives rise to several possibilities; one is, that a soil with but little granulation may become puddled, and a water-logged condition result. This occurred in the case of the Jackson soil where left uncropped. The presence, however, of a crop, even at an early stage of growth, always favored, to an appreciable extent, the passage of water through this soil. For example, a good stand of young wheat nearly always prevented water remaining on the surface to an injurious extent, even during periods of very heavy rainfall. On the other hand, on uncropped cylinders of this soil the water would stand for weeks, especially in the wintertime, if not removed by special means. Another possibility, of rather frequent occurrence, as a mater of fact, is that a sudden downpour of rain in the growing season may furnish little moisture to the crop in the field on account of the run-off, but in the cylinders all will be retained. These considerations lead to the conclusions that the increased moisture supply, at least for well granulated soils, would increase nitrate production, and that this, along with increased leaching, would result in a more rapid loss of nitrogen than would take place under field conditions.

With regard to the effect of the cylinder conditions on the availability of phosphorus and potassium, the writer can say only that those soils which were very poor in these elements failed to respond to the marked extent expected under field conditions.

In conclusion the writer will add that he wishes not to condemn cylinders, and other similar means of conducting experiments in the open, but only to call attention to certain defects and possible sources of error, which need to be overcome in order to make work of this kind comparable with field conditions, at least wherever the rainfall is at all heavy, and the soil of such a nature as to allow of any considerable run-off.

The writer has used covers with apparent good results in the case of a soil of poor granulation—one that was readily puddled—the covers being placed on the cylinders only at times of extra heavy rainfall. Also, the writer will suggest that the problem may be solved by having a vertical slot on one side of the cylinder so as to allow the escape of a certain excess of water, or even

permit of a continuous run-off. Since the soil height is variable, the opening should be adjustable and of easy control, and should be so arranged as to prevent the inflow of water from the outside.

SUMMARY

1. Five different kinds of soil when placed in cylinders sunk in the ground, and exposed otherwise to natural conditions, were found to become unproductive to the extent of complete crop failure in the course of from 3 to 8 years, except where limed. For none of the soils was such a result either observed or to be expected under usual field conditions such as are found in Tennessee.

2. Annual applications of acid phosphate, muriate of potash and nitrate of soda had little or no effect in preventing crop failure. Farmyard manure applied at the outset in moderate and even large amounts delayed crop failure only a year or two in most cases.

3. The only unusual condition apparent to the writer was the projection of the cylinder rims for 2 to 3 inches above the soil surface, thereby preventing all run-off of water in times of heavy rainfall. The increased moisture supply and the excessive leaching were assumed to reduced the natural soil supply of bicarbonate of lime until it became the limiting factor in crop production and finally induced crop failure.

4. The question was raised as to the effect of the cylinder conditions on the availability both of the soil nitrogen and the important mineral elements phosphorus and potassium. The evidence indicated an increased loss of nitrogen and uncertainty with regard to the phosphorus and potassium, the availability of which may have been increased.

5. The suggestion was made that usual field conditions might be approached by providing each cylinder with an adjustable, vertical slot so that either a constant outlet be provided, or that run-off be allowed only at certain times or seasons of the year at the discretion of the operator.

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THE COMPARATIVE RATE OF FORMATION OF SOLUBLE MATERIAL IN CROPPED AND VIRGIN SOILS AS MEASURED BY THE FREEZING-POINT METHOD

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In studies looking toward the maintenance of soil productivity, the importance of information regarding the changes a soil undergoes in passing from a virgin or fertile condition to a semi-productive state has long been recognized. Many chemical methods have been devised to measure such changes, the relative solubility of the soil constituents in water, and mineral and organic acids of different strengths being the basis of comparison. Much valuable information has been afforded by such researches, but no consistent correlation between the crop-producing power of soils and the amount of plant-food elements soluble in any of the reagents tried has been found. Recently Jordan (1) made studies of several soils of different productive power. All the common methods of analysis were made use of, including continuous leaching with water. While there appeared to be some relation between the organic matter extracted by water in one and ten-day periods, respectively and the dry matter of barley produced, the general conclusion was drawn that there was no consistent relation between the crop-producing power of the soils and either their composition as determined by total analysis or the material soluble in any of the solvents used. A review of the available literature shows this conclusion to be in accord with the findings of numerous other investigators.

It was pointed out in a previous publication (2) that the rate of formation of soluble material as measured by the freezing-point method under different conditions of moisture content and temperature was on the whole greater in a virgin than in the corresponding cropped soil. A difficulty experienced in carrying on the experiments referred to was the tendency for the concentration of the soil solution to reach a maximum and decrease within a period of 30 days. This tendency was more pronounced in the case of the virgin than of the cropped soils and consequently the concentration of the solutions tended to approach each other in this time rather than diverge, as might otherwise have been the case.

Experiments conducted with the view of ascertaining the effect of washing soils with distilled water until the concentration of the soil solution was reduced to only a few parts per million, upon the subsequent rate of formation

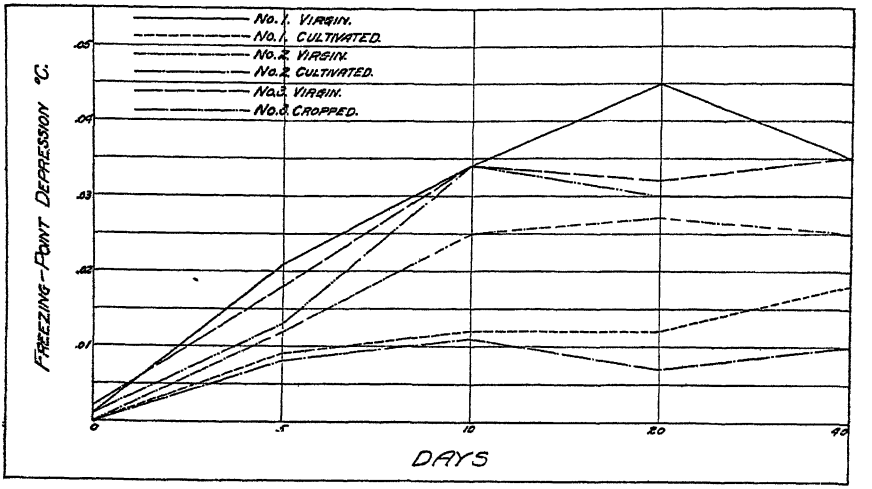

FIG. 1. DIAGRAM SHOWING THE RATE OF FORMATION OF SOLUBLE MATERIAL IN CROPPED AND VIRGIN SOILS

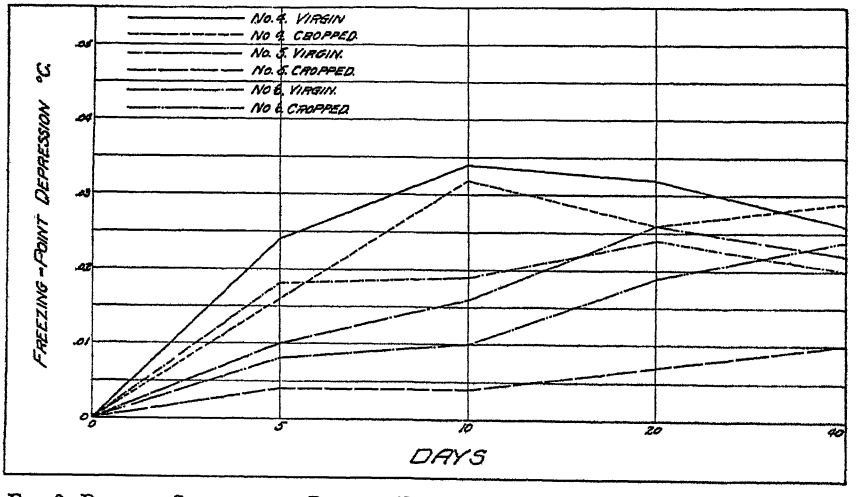

FIG. 2. DIAGRAM SHOWING THE RATE OF FORMATION OF SOLUBLE MATERIAL IN CROPPED AND VIRGIN SOILS

of soluble material, brought out the fact that when soils are so treated the tendency for the concentration of the solution to reach a maximum and then decrease is largely obviated (3). The idea, therefore, suggested itself that if the cropped and virgin soils to be compared were washed as above described and the rate of increase of the concentration of the solution measured, more accurate information regarding the rate of formation of soluble constituents might be obtained.

DESCRIPTION OF SOILS STUDIED

Six soils representing the prevailing types of upland soils in the vicinity of East Lansing were selected for this investigation. Soil 1 is a silt loam of the Miami series and represents the remains of a moraine. It has been under cultivation for 75 years or more but has been handled fairly well. Nevertheless, there has been a marked decrease in its content of organic matter and the crop-producing power has materially changed.

Soil 2 is of the same formation as no. 1. This piece of land has been handled very carelessly and is in an exceedingly poor condition.

Soils 3 and 4 are from low areas surrounded by morainic and till plain formations, respectively. These areas are small in extent and receive considerable wash from the surrounding high land. Both soils are quite high in organic matter and though they have been cropped for many years, their productivity has not appreciably decreased.

Soil 5 is a sand from a till plain area. It has been under cultivation for more than 60 years and is much depleted as a result of tenant farming. The last few years it has been receiving better treatment, and it will be exceedingly interesting to note if any change in the rate of formation of soluble material results from a continuation of this treatment.

Soil 6 is a very fine sandy loam of morainic formation. It belongs to the Miami series and has been under cultivation many years. The system of management has been good and while it is not as productive as originally, it still grows good crops and cannot be classed as a depleted or "run down" soil.

METHOD OF PROCEDURE

Seventy-five grams of air-dry soil were placed on a coarse filter paper in a funnel and washed with distilled water until the soluble material was practically all removed, as indicated by the freezing-point method. When water ceased to drain from the soil, it was thoroughly stirred and samples placed in ordinary freezing-point tubes. By this procedure the soil retained sufficient water so that a short column $\frac{1}{8}$ to $\frac{1}{4}$ inch in height stood over the soil in the tube. The tubes were stoppered and placed in a constant temperature chamber at 25°C. Shortly after the tubes were placed in the chamber the stoppers were removed for a moment to relieve any air pressure which might have developed at the higher temperature. Every few days the contents of the tubes were thoroughly stirred and the tubes allowed to remain unstoppered for a short time to allow the escape of any gas which might have accumulated. The freezing points of the soils were determined in the usual manner after 5, 10, 20 and 40 days. The data obtained are presented in table 1 and in graphic form in figures 1 and 2.

TABLE 1

Freezing-point depressions at different periods of cropped and virgin soils maintained at 25°C.; and the corresponding parts per million of soluble material

SOIL	ORIGINAL		AFTER 5 DAYS		AFTER 10 DAYS		AFTER 20 DAYS		AFTER 40 DAYS	
	Freezing point depressions	Soluble material	Freezing point depressions	Soluble material	Freezing point depressions	Soluble material	Freezing point depressions	Soluble material	Freezing point depressions	Soluble material
		p.p.m.		*p.p.m.*		*p.p.m.*		*p.p.m.*		*p.p.m.*
1. Virgin.....	0.001	25	0.021	525	0.034	850	0.045	1125	0.035	875
Cultivated	0.000	000	0.009	225	0.012	300	0.012	300	0.018	450
2. Virgin.....	0.000	000	0.012	300	0.025	625	0.027	675	0.025	625
Cultivated....	0.000	000	0.008	200	0.011	275	0.007	175	0.010	250
3. Virgin.....	0.002	50	0.018	450	0.034	850	0.032	800	0.035	875
Cropped.....	0.001	25	0.013	325	0.034	850	0.030	750	0.030	750
4. Virgin.....	0.000	000	0.024	600	0.034	850	0.032	800	0.026	650
Cropped.....	0.000	000	0.016	400	0.032	800	0.026	650	0.029	725
5. Virgin.....	0.000	000	0.018	450	0.019	475	0.024	600	0.020	500
Cropped.....	0.000	000	0.004	100	0.004	100	0.007	175	0.010	250
6. Virgin.....	0.000	000	0.010	250	0.016	400	0.026	650	0.022	550
Cropped.....	0.000	000	0.008	200	0.010	250	0.019	475	0.024	600

DISCUSSION

The data presented show that the tendency for the concentration of the soil solution to reach a maximum and decrease before the end of the 40-day period was practically overcome by the washing. Some tendency for the concentration to decrease was exhibited by the virgin sample of soil 1 and by both the cropped and virgin samples of soil 4. The changes, however, were not sufficient to affect the conclusions.

Three of the soils studied, nos. 1, 2 and 5, showed a decidely greater rate of formation of soluble material in the virgin than in the cropped samples. In addition, the total concentration of the soil solution was much higher in the case of the virgin samples. Of the remaining soils two, nos. 3 and 4, showed very little difference in either the rate of formation of soluble material or the total concentration of soil solution attained. It is noteworthy that these two soils are the ones which have shown very little change in productivity as a result of several years' cultivation. Soil 6 showed only a slightly greater rate of formation of soluble material in the virgin than in the cropped sample, the final concentration of soil solution being virtually the same. If the rate of increase of concentration of the soil solution under the conditions used is a criterion of the productivity of soils, one must conclude that this soil is not seriously depleted.

How much of this formation of soluble material is due to biological activities and how much to the direct solubility of the soil constituents, is a question which offers opportunity for much interesting research. This with other problems arising from the work presented is under investigation in the soils laboratories of the Experiment Station.

The writer realizes that the number of soils used in these experiments is small but the data are so in accord with the crop-producing power of the soils that he feels justified in suggesting that a marked decrease in the rate of formation of soluble material is one of the changes a soil may undergo as a result of several years of cultivation with the return of little fertilizing material.

The writer desires to express his gratitude to Dr. M. M. McCool for many helpful suggestions in connection with the above investigation.

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CHANGES IN COMPOSITION OF THE SOIL AND OF THE WATER EXTRACT OF THE SOIL, FOLLOWING ADDITION OF MANURE

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These experiments were begun in the hope of discovering the effect of the addition of manure to the soil on the composition of the soil solution. This knowledge was sought for the purpose of devising a method for the rational evaluation of farm manures. The data obtained are thought to contain matter of value for the adequate understanding of the effects of manure in the soil; and also to have some practical bearing on the evaluation of manure.

DESCRIPTION OF THE FIRST EXPERIMENT

Several pounds of well-mixed fresh horse manure, consisting of wet droppings and litter, were made to pass a 10-mesh sieve by being ground through a sausage mill, and the mass again well mixed. The desired quantities were weighed out and added to weighed amounts of air-dry soil placed in 1-quart glass Mason jars. The whole was well mixed by shaking and allowed to stand for 2 hours; then the contents were passed through a 10-mesh sieve, again mixed and returned to the jars. Water was added to bring the moisture content of the mixture to 22 per cent. The jars were kept in a store-room at a temperature of 15° to 20°C. From time to time one jar of each mixture was used for analysis. Six jars of each composition were prepared. The soil used was the Yolo silty clay loam, air-dry, well sifted and mixed. After 254 days, when the last jars had been used for analysis, the remaining contents of all the jars of each mixture were mixed together and returned to a single jar; water was added to make 22 per cent; and then the contents set aside for 145 days longer, when they were analyzed. Each time the soil and manure mixtures were analyzed, a portion of the original manure was analyzed by similar methods, except that a 1 : 10 water extract of the manure was made instead of the 1 : 5 extract which was made of the soils. Three different proportions of manure were used in the soil, so that at each time analyses were made, there were 4 samples, one of the soil alone, then a 1 per cent, a 2 per cent and a 5 per cent mixture of manure with the soil. All

[1] Grateful acknowledgment is herewith made for valuable suggestions given and criticism offered by Professors John S. Burd and D. R. Hoagland, also to Mr. A. W. Christie and Mr. J. C. Martin for much of the analytical work.

data here given are from the first experiment. One per cent is equivalent to about 20 tons per acre. The methods of analysis used were in general those described by Stewart (11). The total carbon was determined by wet combustion. Total nitrogen was determined by the Gunning copper sulfate method.

DESCRIPTION OF THE SECOND EXPERIMENT

The analytical results from the first experiment were so unexpected that a second experiment was planned with the hope of overcoming difficulties which it was feared had vitiated the first experiment. The previous experiment showed much less than the expected increase of soluble matter in the soil extract due to the addition of manure, and a preliminary loss of nitrate followed by a considerable increase later. Although there was in the latter part of the experiment a gain of soluble matter, it never nearly equaled the sum of that in the soil plus that added in the manure. It was feared that an insufficient supply of oxygen was at least partly responsible for these effects. In the second experiment it was sought to avoid this possibility by using 5-gallon (11 inches in diameter, 12 inches deep) stoneware jars containing 50 pounds of soil. The jars were buried in the open ground nearly to the tops and exposed to all weather but protected from rain. Two soils were used, the Yolo silty clay loam, and the Madera sandy loam, but only one mixture of each was made, namely 2 per cent manure with the soil.[2] The mixtures were prepared very much as for the first experiment, being mixed dry, then placed in the pots and water added to bring to optimum moisture conditions. On account of exposure to the weather, this experiment was not as satisfactory as the first. It was necessary to add large amounts of water to maintain the proper moisture content. The addition of this water at the top of the soil eventually partially puddled the soil and apparently caused some translocation of soluble matter to the tops of the jars, due to the constant evaporation from the tops. After the experiment had continued for 3 months, this great loss of water was largely prevented by keeping the jars loosely covered.

The results were disappointing. Due to the great variation in moisture between the tops and bottoms of the jars and to the addition of much water to make up the loss by evaporation, it was found that the chemical and biological changes taking place in the soils were considerably interfered with. Nevertheless, the analytical results are quite similar to those of the first experiment, and confirm them. Primary decrease of soluble matter and loss of nitrates due to the addition of manure was followed by a gradual increase to much above that in the control soils.

None of the figures and tables here reported are taken from the results of the second experiment.

[2] The possibility that different soils would give different results, was the reason for using two quite different soils in the second experiment. A large difference in level of the curves for nutrients in the two soils was observed, but the curves were relatively parallel throughout, indicating similar effects consequent on addition of manure.

CARBON DIOXIDE IN THE SOIL ATMOSPHERE

It was expected that biochemical action in the soil manure mixtures would release much carbon dioxide which would increase the mineral content of the soil solutions. At the time of starting the first experiment a set of similar mixtures was made and placed in bottles so arranged that the air within them could be drawn off in order to determine its content of CO_2. Evolution of the gas was most rapid at the beginning as soon as moisture equilibrium was attained, and gradually decreased as time went on. The amount of carbon dioxide found was somewhat proportional to the amount of manure in the mixture, but it seemed to have no relation to the minerals in the soil extract (see tables 1 and 4). Similar results as to evolution of carbon dioxide have

TABLE 1

Per cent CO_2 *in air of soil-manure mixtures*

TIME	MIXTURES			
	1 per cent	2 per cent	5 per cent	10 per cent
days				
0	12.7	13.4	26.8	28.2
1	6.2	11.4	24.6	24.5
4	15.6	22.0	32.3	37.2
6	10.7	16.8	31.2	32.8
9	11.2	18.2	31.7	35.2
18	9.5	19.8	25.8	38.8
26	8.4	15.1	22.9	36.0
31	6.1	11.8	18.7	33.6
38	7.7	13.1	22.1	35.2
45	5.8	9.9	17.0	27.0
52	5.8	9.8	15.3	30.0
59	5.8	9.6	14.6	28.3
66	5.0	8.8	13.6	28.2
73	3.6	6.6	10.0	22.1
80	3.1	6.0	9.6	21.3
87	2.7	6.5	11.1	24.0
94	2.9	5.6	8.8	17.0
101	3.2	5.6	8.9	19.0

been reported by Potter and Snyder (7), Merkle (6), and Russell and Appleyard (8). The last-named authors found increased NO_3 somewhat proportional to CO_2 in soil air, but did not consider other mineral matter as affected by CO_2. Thus it appears that liberation of much CO_2 is not necessarily followed by great increase of solubility of soil minerals.

TOTAL CARBON

The total carbon in the soil-manure mixture decreases rapidly with time and in proportion to the amount of manure added, so that after one year the amounts remaining in the different mixtures are much more nearly alike

than at the beginning. From the manure by itself, if it is kept moist and cool, there is a large loss of carbon. This may make it appear that there has been water added because water less easily escapes in gaseous form than CO_2. As a consequence of the loss of carbon the proportion of ash becomes greater in the remaining material. Under the best conditions there should be little loss in nitrogen, so that the manure appears to become richer in plant-food as it gradually rots away. This is a principal reason why old manure is more valuable than fresh (see table 2).

TABLE 2

Nitrogen and carbon in soil-manure mixtures. Expressed as per cent of dry substance

DAYS AFTER MIXING	PROPORTION OF MANURE ADDED TO SOIL							
	None		1 per cent		2 per cent		5 per cent	
	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon	Nitrogen	Carbon
0	0.132	1.42	0.137	1.51	0.139	1.63	0.155	2.03
11	0.137	1.36	0.137	1.44	0.150	1.50	0.156	1.83
40	0.133	1.38	0.138	1.46	0.141	1.53	0.156	1.80
90	0.134	1.38	0.138	1.45	0.140	1.50	0.156	1.71
160	0.135	1.37	0.142	1.43	0.148	1.46	0.159	1.62
254	0.135	1.34	0.140	1.35	0.152	1.41	0.158	1.55
395	0.132	1.29	0.136	1.33	0.139	1.39	0.152	1.49

TOTAL NITROGEN AT VARIOUS TIMES

In these experiments the total nitrogen in the mixtures has not varied from first to last within the limits of accuracy of the method of analysis. In the manure itself there was an increase of the per cent of nitrogen due to the loss of carbon as above mentioned. As shown by Russell and Richards (9), loss of nitrogen gas from manure occurs only when there is alternation

TABLE 3

Water-soluble solids in manure and soil-manure mixtures. Expressed as parts per million of dry substance

DAYS AFTER MIXING	MANURE (UNMIXED)		PROPORTION OF ADMIXED MANURE							
			None		1 per cent		2 per cent		5 per cent	
	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile
0	125,920	80,280	318	223	449	295	515	374	874	585
11	146,080	100,670	366	173	508	247	438	256	699	425
40	149,330	104,400	414	174	401	201	454	153	569	301
90	134,000	85,320	695	311	578	305	659	343	683	362
160	130,760	79,248	624	309	748	357	769	351	1,024	502
254	118,900	72,400	863	436	1,080	552	1,205	552	1,610	724
395	103,740	45,360	804	436	910	523	940	529	1,410	705

TABLE 4

Composition of the water extracts of manure and soil-manure mixtures. Expressed as parts per million of dry substance

	DAYS AFTER MIXING	MANURE ALONE	PROPORTIONS OF ADMIXED MANURE			
			None	1 per cent	2 per cent	5 per cent
Ca	0	6,408	38.2	41.7	54.7	59.1
	11	7,227	49.9	43.4	37.1	37.7
	40	7,474	73.5	76.9	66.7	65.1
	90	6,420	69.5	69.7	70.6	71.7
	160	5,715	46.0	46.3	46.4	53.5
	254	7,830	41.8	48.6	49.0	60.2
	395	2,184	51.0	51.0	51.0	70.0
Mg	0	1,224	18.1	21.5	22.8	32.8
	11	935	20.3	18.7	19.6	28.1
	40	755	20.3	23.8	21.3	20.9
	90	701	23.5	22.3	24.5	25.0
	160	1,025	24.9	26.2	33.8	38.8
	254	2,160	42.5	51.8	46.9	56.3
	395	1,268	36.0	46.0	49.0	54.0
K	0	12,325	28.0	28.8	36.1	59.1
	11	12,833	34.6	45.4	42.5	97.3
	40	13,020	22.7	20.7	34.7	43.8
	90	13,062	36.4	37.2	37.6	42.3
	160	13,908	44.7	62.9	70.3	72.9
	254	13,950	42.5	42.8	54.5	91.8
	395	14,154	49.0	60.0	55.0	79.0
SO_4	0	2,711	21.6	28.2	34.8	60.4
	11	3,066	31.3	31.4	35.0	56.9
	40	3,001	19.4	30.1	23.3	28.8
	90	2,428	18.5	29.2	39.0	45.8
	160	2,698	27.6	36.4	42.4	61.5
	254	1,658	62.5	35.0	43.0	67.3
	395	1,138	48.0	60.0	69.0	105.0
PO_4	0	183	5.1	5.1	7.1	8.8
	11	187	4.5	6.7	4.0	10.1
	40	7,918	6.7	11.4	11.3	11.6
	90	6,900	5.3	7.3	8.1	12.3
	160	6,361	5.3	4.6	8.0	15.4
	254	5,380	4.7	5.2	6.8	8.3
	395	2,684	5.0	12.0	9.0	12.0
NO_3	0		26.1	28.8	27.0	23.7
	11		55.9	8.0	4.7	4.8
	40		101.5	65.6	22.7	8.9
	90		115.2	86.3	67.2	43.7
	160		147.0	151.0	145.0	193.0
	254		286.0	324.0	345.0	413.0
	395		317.0	368.0	360.0	560.0

TABLE 4—*Continued*

	DAYS AFTER MIXING	MANURE ALONE	PROPORTIONS OF ADMIXED MANURE			
			None	1 per cent	2 per cent	5 per cent
Freezing-point depression.....	0					
	11		0.051	0.051	0.061	0.106
	40		0.061	0.074	0.068	0.101
	90		0.085	0.084	0.081	0.139
	160		0.083	0.098	0.100	0.147
	254		0.058	0.094	0.118	0.212
	395		0.124	0.127	0.168	0.221

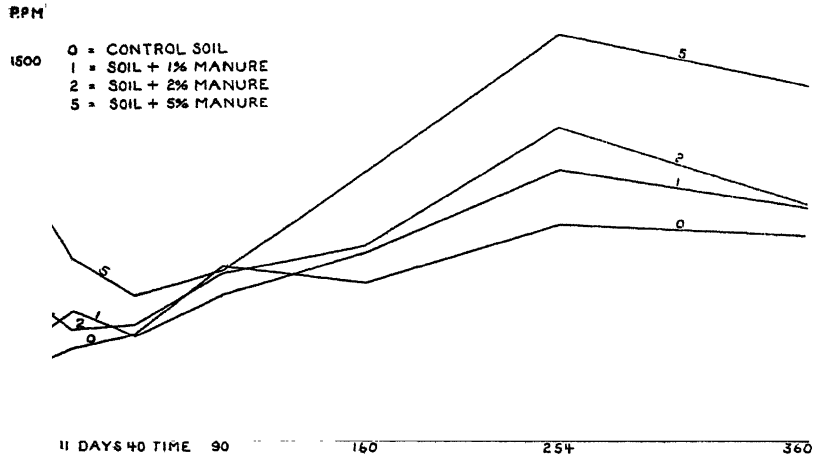

FIG. 1. DIAGRAM SHOWING TOTAL SOLIDS IN WATER EXTRACTS

of aerobic and anaerobic conditions. In the present experiments aerobic conditions were continuous, hence no loss of nitrogen was to be expected. No attempt was made to determine in what form the nitrogen existed in the mixtures, except to follow the changes in nitrate. No doubt there were considerable changes in the form of combination of the nitrogen, as argued by Sabashnikov (10). Even there may have been some loss of gaseous nitrogen with corresponding fixation by *Azotobacter*, as indicated by Hutchinson (2), but the total remained nearly constant (see table 2).

TOTAL MATTER SOLUBLE IN WATER EXTRACTS AT VARIOUS TIMES

There was in general an almost immediate notable decrease of soluble matter after addition of manure to the soil, and greater in proportion to the amount of manure added (see figure 1 and tables 3 and 4). This is a striking phenomenon when one considers that there was added in the manure more soluble matter than the soil already contained. Whether this fixation was due to physical, chemical or biological action is the subject of further

investigation. This decrease of soluble matter was considerable the day after the mixtures were made. That is, the sum of that contained in the soil and in the manure was considerably less than was actually found in the mixture (see figure 2 and table 5). After 40 days the soluble matter in the 1 per cent mixture was actually less than in the control soil. But after 160 days the reverse was true. Still at no time within 395 days was the amount of soluble matter in the mixtures equal to the sum of that found in the control soil plus that in the manure added.

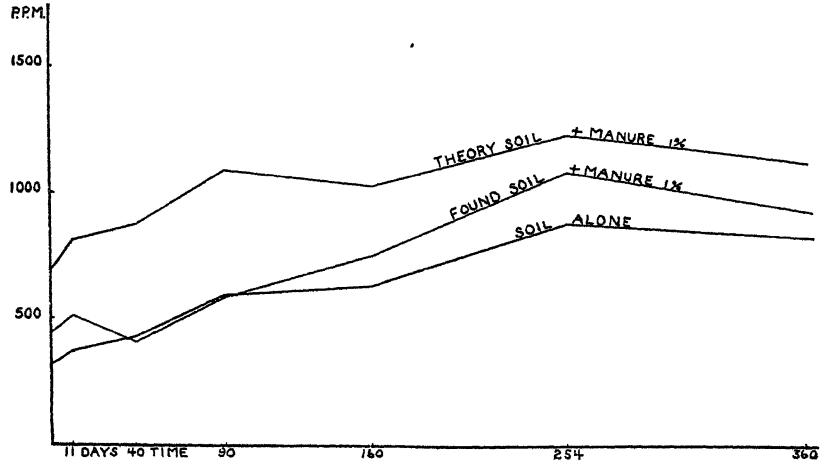

FIG. 2. DIAGRAM SHOWING FIXATION OF WATER-SOLUBLE MANURE EXTRACT BY SOIL

This fixation of soluble matter varies largely for the different ions and complexes. A large proportion of the organic matter as indicated by loss on ignition, and practically all of the potassium were fixed, also much of the calcium. Little of the magnesium and almost none of the sulfate and phosphate were fixed. A study of the annexed tables and graphs will make this more clear. These all refer to the first experiment. Very similar results were obtained in the second experiment under outdoor conditions, but on account of complications due to excessive variations in moisture, the figures are less concordant. These figures are not given, as it seemed that they shed no different light on the subject.

All these observations refer only to the water extracts, and it is not implied that exactly proportional changes necessarily take place in the real soil solution. Hoagland (1) has discussed the relationships between the water extracts and the true soil solution.

FIXATION

Fixation as above mentioned has also been observed by White (14) in working with green manures (cover crops). He explains the fixation as due to biochemical action which destroyed organic acids, liberating the mineral bases which were combined with them, thus increasing alkalinity and decreas-

ing water-soluble plant-food. Jensen's (3) experiments were not parallel with these here reported but in some respects his results confirm them.

The real cause of this fixation is still a subject of speculation.[3] In the present investigation the analytical data obtained are apparently inadequate to explain it. The nitrate of the soil was quickly used up after the addition of manure, but it was too small in amount to account for much increase in alkalinity. It seems hardly possible that biochemical action could produce such effects in so short a time. Perhaps it may be due to both chemical and physical action or to one or the other alone. After some months when considerable nitrate was formed, one would expect increased solubility of bases as was found.

TABLE 5

Theoretical compared with actual total solids in water extract from soil-manure mixture. One per cent fresh manure added to air-dry soil

TIME	PARTS PER MILLION IN DRY SUBSTANCE				FOUND PER CENT OF THEORY
	Manure	Soil	Sum	Found	
days					
0	377	318	695	449	64
11	438	366	804	508	63
40	447	414	861	401	46
90	402	695	1097	578	53
160	390	624	1014	748	73
254	357	863	1220	1080	89
395	309	804	1113	910	82

Figures given express the amounts found at each of the several sampling periods as follows: Manure = amount added to soil by 1 per cent manure; Soil = amount found in control soil; Sum = arithmetical sum of manure + soil; Found = amount found in the mixture; Found per cent of theory = per cent of sum actually found.

TABLE 6

Fixation of plant-food by soil

	PARTS PER MILLION IN DRY SUBSTANCE			
	M.	S.	S. + M.	Found
Total solids	377.0	318.0	695.0	449.0
Loss on ignition	240.0	223.0	463.0	295.0
Ca	19.0	38.0	57.0	42.0
Mg	4.0	18.0	22.0	22.0
K	37.0	28.0	65.0	29.0
SO_4	8.0	22.0	30.0	28.0
PO_4	0.5	5.1	5.6	5.1

M. = amount added in manure; S. = amount found in soil; S. + M. = sum of the two; Found = amount actually found in mixture one day after mixing.

[3] In another set of experiments, now in progress, in which the same manure, well rotted, was used with the same soil, very different results are being obtained. There is no loss of nitrate and less fixation of other ions than in the first experiment.

CHANGES IN NITRATE

Within one day after making the mixtures of soil and manure for the first experiment, nitrate had practically disappeared (see fig. 3). In the second experiment its disappearance was somewhat slower, possibly on account of better aeration. A gain of nitrate began after 40 days, and was most rapid in those mixtures having most manure. After 160 days nitrate in the mixture was proportional to the amount of manure added, and exceeded that in the control soil. Denitrification, loss of nitrogen as gas, has been shown by Russell and Richards (9) to occur only under alternating conditions of excess

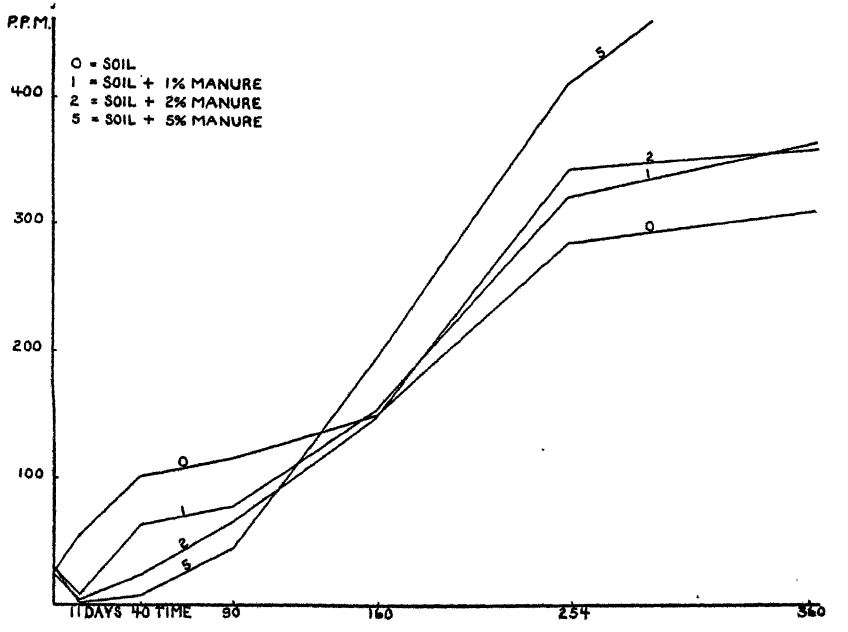

FIG. 3. DIAGRAM SHOWING NO_3 IN SOIL AND MIXTURES

or deficiency of oxygen. In these experiments it is probable that there was never a deficiency of oxygen so that that cause of loss of nitrate cannot be assumed. Others (2, 9, 10, 14) have made similar observations.

It seems most probable that the initial loss and later gain of nitrate are due chiefly to biochemical activity (14). The practical problem is so to handle and apply manure to the soil that the nitrate will be increasing at the time when the crop to be grown needs it. The primary fermentation resulting in the decrease of nitrate should have been completed before the manure is applied to the soil, so that after the mixture is made, nitrification will soon begin if it is desired to stimulate a growing crop. This explains why well rotted manure is so much esteemed by farmers. On the other hand, it may be better to apply raw manure in the latter part of the growing season in

order to furnish energy for *Azotobacter* which will thus be enabled to fix atmospheric nitrogen while the nitrogen of the manure is gradually becoming available for the next season's crop.

CHANGES IN THE COMPOSITION OF MANURE DUE TO ROTTING

The most notable of these changes is the loss of carbon with the resultant gains in the percentage of other constituents. In some cases it has been observed that there was an actual increase of moisture in the rotted manure which would indicate that the CO_2 escaped more rapidly than water (see table 7).

TABLE 7

*Percentage composition of manure used in experiment 1 at each sampling period on the water-free basis**

TIME	ASH	ORGANIC	NITROGEN	TOTAL CARBON
days				
0	15.3	84.7	1.55	41.9
11	15.7	84.3	1.55	42.1
40	15.5	84.5	1.60	42.0
90	15.8	84.2	1.56	42.0
160	16.7	83.3	1.57	42.0
254	17.6	82.4	1.72	41.3
395	18.2	81.8	1.88	41.1

* The original manure at time of mixing contained 70.3 per cent water, 0.305 total P_2O_5; and 0.424 total K_2O.

CHANGES IN THE COMPOSITION OF THE MANURE EXTRACT

Soluble organic matter in the manure decreases as rotting proceeds as might be expected, since this soluble matter is composed of the most easily fermentable constituents, but the soluble mineral matter increases as rotting proceeds, though after 3 months some of the ions began to decrease. Notable increases are in soluble phosphate and ammonia. These changes are apparent in table 8. The decrease in soluble Ca and Mg is probably due to the increase in alkalinity from the change of organic nitrogen to ammonia. Soluble sulfate (SO_4) decreased somewhat regularly, perhaps because of changing from organic to inorganic combination.

CHANGES IN ALKALINITY

Changes in hydrogen-ion concentration caused by the change of organic nitrogen, phosphorus and sulfur to their mineral acid forms are probably adequate to account for the increased solubility of calcium, magnesium and potassium as pointed out by White (14). When changed from the ammonia form to the nitrate form, 14 pounds of nitrogen would release acid enough to neutralize

100 pounds of calcium carbonate. Since the nitrogen is present largely in organic combination at first, when it changes to ammonia, a notable increase of alkalinity must occur. On changing to nitrate, this alkalinity would be replaced by an equal acidity which would displace CO_2 and perhaps silicic acid with the formation of soluble nitrates of calcium, magnesium, sodium and potassium. Phosphorous and sulfur when oxidized would greatly increase acidity. Though these changes in acidity appear to be theoretically probable, no determinations were made in order to follow them.

TABLE 8

Composition of manure extract from the same sample at successive sampling periods

TIME	PARTS PER MILLION REFERRED TO WATER-FREE SUBSTANCE							
	Total solids	Loss on ignition	Ca	Mg	K	N as NH_3	SO_4	PO_4
days								
0	125,920	80,280	6,408	1,224	12,325	340	2,711	183
11	146,080	100,670	7,227	935	12,833	1,740	3,066	187
40	149,330	104,400	7,474	755	13,020	1,740	3,001	7,918
90	134,000	85,320	6,420	701	13,062	2,690	2,428	6,900
160	130,760	79,248	5,715	1,025	13,908	4,210	2,698	6,361
254	118,900	72,400	7,830	2,160	13,950	5,100	1,658	5,380
395	103,740	45,360	2,184	1.268	14,154	4,410	1,138	2,684

INCREASE OF PLANT-FOOD

Russell and Appleyard (8) have shown that after temperature and moisture, which are most important, the chief factors governing the increase of plant-food in soils are, numbers of bacteria, nitrate and carbon dioxide which run parallel. Tottingham (13) found an increase of nitrogen due to the addition of straw to manure and assumed it to be caused by the extra supply of energy furnished by straw to the nitrogen-fixing bacteria. Jensen (3) found increase of plant-food due to the action of mineral-free organic solvents. Organic matter with calcium carbonate gave a greater increase than without calcium carbonate on sandy soil; on clay soil the reverse was true. Lipman and Blair (4) and Potter and Sndyer (7) have also obtained some such results. Thorne (12) has maintained that the value of manure lies almost solely in the additional plant-food which it furnished the soil. If the gain of water-soluble mineral matter following the application of manure is simply additive, his position seems to be well taken. The present experiments, as shown by table 4, show that the effect of fresh manure is not simply additive, but that the soluble matter is less than the sum of that in the soil and in the added manure. It is known that *Azotobacter* may fix atmospheric nitrogen when supplied with a carbohydrate source of energy. In this way the soil would be enriched by the addition of manure by more than the

amount of plant-food in the manure. In these experiments there is no evidence of such fixation of nitrogen. Also, it might be expected that after the addition of manure, the increased acidity due to the change of nitrogen to nitrate, phosphorous to phosphoric acid and sulfur to sulfate would increase Ca, Mg, Na and K in the soil solution. Results of these experiments do not indicate such effects. They offer no ground for disputing the correctness of Thorne's position, that manure is valuable chiefly or solely for the plant-food it contains. As to the correctness of Thorne's position, the writer does not here offer any opinion.

On the other hand, it may be pointed out that considering the short duration of the present experiments, and the small increase in nitrate, together with the practically constant total nitrogen, the manure had not yet had time to produce its most notable and valuable effects. General experience and notably the historic experiments at Rothamsted support the view that the effect of manure, especially when applied in large amounts, is only gradually apparent and may endure for some years.

SUMMARY

1. Fresh manure was mixed with soil in the proportions of 1, 2 and 5 per cent, the mixtures kept in loosely covered glass jars at optimum moisture, and analyzed from time to time with the following results.

2. Carbon dioxide in the soil atmosphere was increased in proportion to the manure added.

3. Total carbon gradually decreased and more rapidly for larger amounts of manure.

4. Total nitrogen was constant from the first to the last of the experiment.

5. Total water-soluble matter was within a few weeks decreased in the 1 per cent mixture to below that in the control soil. After that it gradually increased to considerably above that in the control soil, but never to equal the sum of that in the control soil plus that in the added manure. The 2 and 5 per cent mixtures showed relatively greater decreases and increases.

6. Further investigation of the cause of the observed fixation is necessary in order to explain it adequately.

7. These changes were not relatively the same for the different ions and complexes.

8. In the soil extract, there was less change of Ca and Mg than of K, SO_4 and NO_3.

9. Nitrate at first quickly disappeared, and later increased considerably over that in the control soil. These changes are ascribed to biochemical action.

10. Increase in water-soluble mineral matter after the addition of manure to soil can be accounted for largely by that derived from the mineral matter of the manure itself.

CONCLUSIONS

1. When fresh manure is mixed with soil the resulting reaction decreases the amount of water-soluble plant-food, so that crops started at that time might be retarded, or at least not immediately benefited by the manure. When the manure becomes well decomposed it adds materially to available plant-food. Consequently, fresh manure should not be added to a soil just previous to or during the time a crop is growing.

2. If the soil is kept sufficiently open to the air, there should be no loss of nitrogen due to the addition of manure.

3. Liberation of large amounts of CO_2 in the soil by rotting manure does not always greatly influence the composition of the water extract of the soil.

4. The water-holding capacity and porosity of soil are undoubtedly improved by manure, quite apart from increase in soluble plant-food, but the latter is increased chiefly or solely by the amount added in the manure, and very little due to reactions set up by the manure in the soil.

5. The conclusions here drawn are strictly applicable only to these experiments. Since the conditions were quite unlike those obtaining in the field, it may be possible that field experiments would show quite different results.

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THE COMPARATIVE VALUE OF VARIOUS FORMS OF LIMESTONE

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The beneficial effect of the use of lime has been fully realized in America only within the last few years. Some of the older experiment stations, among them Pennsylvania, Maryland, and Rhode Island, were the first to demonstrate the benefit derived from liming the soil, and both Pennsylvania and Maryland also emphasized the feasibility of using ground limestone for this purpose. The work of the Illinois Experiment Station has demonstrated the great value of ground limestone on acid soils. There are some questions, however, regarding the use of limestone upon which additional information is needed: viz.,

1. Can dolomitic limestone be used successfully on acid soils, and what is its value compared with high-calcium limestone?
2. What is the comparative value of the finely ground material and that more coarsely ground, which can be purchased more cheaply?
3. What is the durability of the various forms and kinds of lime when applied to soils in the field?
4. What effect does the application of limestone to the surface have upon the acidity of the subsurface and the subsoil?
5. What is the annual loss of limestone from the soil, and what are the factors which contribute to this loss?

COMPARATIVE EFFECT OF HIGH-CALCIUM AND DOLOMITIC LIMESTONE

Since 1912, the experimental farm at Newton, Jasper County, Illinois has been devoted primarily to a study of these questions.

The comparative value of high calcium and dolomitic limestone was studied on 114 plots on this farm by applying both kinds of limestone in varying amounts and degrees of fineness, including burnt lime. This experimental farm was established in 1912. In 1916, three and a half years after the initial application of limestone to the soil, samples of the soil were collected and submitted to careful chemical analysis for residual limestone and acidity.

The results, obtained by averaging the data from all plots, 15 plots in each case receiving equivalent applications of the same kind of limestone irrespective of the degree of fineness, are recorded in table 1.

The amount of residual limestone found was, as an average, distinctly larger where dolomitic limestone had been added; that is, this form of stone was more lasting in the soil. However, notwithstanding its more lasting quality, it was fully as effective in destroying the soil acidity as was the high-calcium limestone. In every case where high-calcium limestone had been applied, the average results showed a smaller amount of residual limestone, a larger annual loss of limestone, and less acidity destroyed. The data also show that the larger the amount of limestone applied, the more residual carbonate was found, the more acidity was destroyed, and the larger was the loss of limestone from the surface soil.

TABLE 1

Comparative effect of high-calcium and dolomitic limestone (including burnt lime)—Newton Field, 1916

Average pounds of calcium carbonate in 2,000,000 pounds of surface soil (one acre about 0 to 6⅔ inches deep)

SERIES	AVERAGE OF ALL SERIES			
Lime applied	High calcium	Dolomitic	High calcium	Dolomitic
	Limestone found		Annual loss	
	pounds	*pounds*	*pounds*	*pounds*
Light application	499	725	392	304
Medium application	1,095	1,330	849	777
Heavy application	3,005	4,035	1,599	1,258
Average annual loss			946	779
Acidity destroyed				
Light application	628	708		
Medium application	936	947		
Heavy application	1,396	1,562		
Net average annual loss			967	554

EFFECT OF DEGREE OF FINENESS

The effect of the degree of fineness of limestone upon the loss of limestone and upon the acidity in the surface soil is shown by a study of the results found in table 2. The figures are obtained by averaging the data from all plots which received like applications of both the high-calcium and the dolomitic limestone. (The results are the averages of six separate determinations from as many separate plots.)

In general, the finer the stone, the greater was the loss of limestone. The mill-run stone (¼ inch down) was practically as effective as any grade in destroying the acidity and in addition possessed better lasting qualities. The fine material present in this grade of stone seems to be sufficient for the

immediate requirements of the soil, and the residual properties are of value in maintaining an alkaline reaction in the soil. An important point brought out clearly in the above table is that the annual loss of limestone is dependent upon the amount of limestone applied to the surface. The loss of limestone is roughly proportional to the amount applied and varies only from 14 to 16 per cent of the total amount applied to the surface.

TABLE 2

Effect of degree of fineness of limestone

Average pounds of calcium carbonate in 2,000,000 pounds of surface soil (one acre about 0 to 6 inches deep)

	FINENESS				
	½ inch down	½ to 1/10 inch	1/10 inch down	1/50 inch down	Burnt lime
Limestone found					
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Light application	617	1,151	631	272	392
Medium application	1,613	2,117	1,025	659	648
Heavy application	3,899	4,776	3,558	3,545	1,822
Acidity found					
Light application	289	356	322	450	395
Medium application	437	222	367	468	348
Heavy application	75	64	110	136	56
Acidity destroyed					
Light application	631	620	709	637	746
Medium application	863	1,059	925	855	1,006
Heavy application	1,368	1,443	1,458	1,492	1,634
Average annual loss of limestone					
Light application	358	208	331	482	385
Medium application	721	523	871	995	953
Heavy application	1,351	1,080	1,419	1,417	1,869

EFFECT OF LIMESTONE ON ACIDITY OF SUBSURFACE

The results from the Newton experimental field are not conclusive in showing any marked effect upon the acidity of the subsurface. The limestone applied to the surface undoubtedly does have some effect in neutralizing the acidity of the subsurface, but it is an exceedingly slow process and requires the lapse of considerable time after the initial application of limestone to the surface. The complete destruction of the acidity even in the surface soil is a slow process since even the heavier applications of limestone upon the Newton field after the lapse of three and a half years had not been sufficient to destroy completely all the acidity in the surface soil. This is undoubtedly due to the difficulty of thoroughly mixing the surface soil and

the applied limestone under ordinary systems of cultivation, such as are generally practiced on the average farm.

The effect of the limestone applied to the surface soil upon the destruction of the acidity of the subsurface is shown clearly by the results obtained from the Odin experimental field. Samples of soil were taken from this field 14 years after the initial application of limestone. It was found that all the acidity of the surface had been destroyed except in one or two instances; also that the acidity of the subsurface had been appreciably decreased by the application of limestone to the surface. The effect of the applied limestone on the acidity of the subsurface is recorded in table 3.

TABLE 3

Effect of limestone on subsurface soil. Averages of all plots, series 100 to 400: Odin Field, 1917

Average pounds of calcium carbonate in 4,000,000 pounds of subsurface soil (one acre about 6⅔ to 20 inches deep)

NUMBER OF PLOTS	LIME ADDED TO SURFACE SOIL	LIMESTONE FOUND	ACIDITY		LIMESTONE NET GAIN FOR SUBSURFACE
			Found	Destroyed	
		pounds	*pounds*	*pounds*	*pounds*
16 plots	None	None	3,703		
24 half-plots	Light application	451	2,735	968	1,419
24 half-plots	Heavy application	1,083	1,834	1,869	2,952

As a result of the application during 14 years' time the sum of the limestone destroyed in neutralizing acidity in the subsurface and that found remaining in that stratum amounted to 2952 pounds where the heavy application had been made and 1419 pounds where the light applications had been made. These figures represent an annual gain of 211 pounds and 94 pounds of limestone, respectively, in the subsurface stratum. If these numbers be subtracted from the annual loss from the surface soil, the unaccounted annual loss from that stratum amounts to 601 pounds and 484 pounds, respectively, for the heavy and the light applications.

THE EFFICIENCY OF A TON OF LIMESTONE

The amount of limestone which may be economically added to an acre at any one time is limited. Two questions, therefore, of very great importance to the farmer are: (1) What is the maximum amount which may be economically used? (2) What becomes of the applied limestone and how long does it last in the soil? The data presented show that the annual loss of limestone is dependent upon the amount added. When the ¼-inch mill-run product was used the annual loss was 14 per cent of the application. From the data presented in table 2 the rate of the loss of limestone for the various applications may be readily calculated. Such calculations are made for the two kinds of limestone used and are recorded in table 4.

The data from the Odin field clearly show that 1 ton of limestone applied every 4 years maintained the soil in an alkaline condition. This fact, together with the data presented in table 4, seems to indicate that this rate of application is the more desirable one to use after the initial acidity has been destroyed.

TABLE 4

Residual limestone in treated soil

RATE OF APPLICATION	REMAINING AT THE END OF					
	First year	Second year	Third year	Fourth year	Fifth year	Sixth year
High calcium limestone						
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1 ton per acre	1,184	870	556	242		
2 tons per acre	2,572	1,893	1,214	535		
4 tons per acre	5,604	4,325	3,046	1,767	488	
Dolomitic limestone						
1 ton per acre	1,191	948	705	462	219	
2 tons per acre	2,619	1,998	1,376	754	132	
4 tons per acre	5,744	4,738	3,732	2,726	1,720	714

CONCLUSIONS

1. From the evidence thus far secured, it appears, that for the common farm land of acid regions, an application of 1 ton per acre of limestone once in 3 or 4 years is sufficient to keep the soil alkaline, or sweet, after the initial acidity has been destoyed by heavier applications.

2. Dolomitic limestone can be used successfully on acid soils. It is slightly more effective than high-calcium limestone in neutralizing the soil acidity, is more durable, and has no injurious effects on the crop yields.

3. As the result of nearly 4 years' work on the Newton field, there is no evidence that finely ground limestone is more effective in correcting soil acidity than is the total product from a $\frac{1}{4}$-inch screen, which contains both the finer material for immediate use and the coarser material for greater durability. This "mill-run" product seems to be the most economical form to use; but final conclusions must await further data concerning crop yields.

4. The destruction of the acidity of the soil is not a rapid process, for it is dependent upon the complete mixing of the applied limestone with the surface soil, which is a slow process. Limestone applied to the surface slowly penetrates into the subsurface. This process, however, requires considerable time. On the Odin field after 14 years, one-half the acidity in the subsurface was neutralized where the larger applications had been made to the surface, and one-fourth where the lighter applications had been made. Applications of limestone to the surface soil seem to have no effect upon the acidity of the subsoil. The amount of native limestone found in the subsoil is a

variable quantity. In some cases there is none present even at a depth of 40 inches, whereas in other cases it extends upward even slightly into the subsurface.

5. The annual loss of limestone from the soil depends upon a number of factors, among which are the kind, the form, and the amount added. The data presented show that the annual loss of limestone is not so large as generally assumed. As an average of all determinations, the annual loss from the surface 20 inches was 760 pounds per acre from the Newton field and 542 pounds per acre from the Odin field. A study of the total calcium indicates that the actual loss of bases may have been less than is shown by these figures, which are based upon the carbon dioxide and acidity determinations.

6. It is very evident from the data presented that chemical analysis may be depended upon to measure the acidity in the soil, the reduction in acidity due to the action of limestone applied, and also to find the limestone still remaining in the soil, whether from applications made or from a supply native to the soil.

LIME REQUIREMENTS AS DETERMINED BY THE PLANT AND BY THE CHEMIST[1]

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The soil used represented the surface soil taken to the depth of plowing. Some of that taken in June, 1916, was sent upon request to F. P. Veitch, then referee on soils for the Association of Official Agricultural Chemists. He planned to submit it in part for coöperative work on chemical methods for determining "lime requirements."

The results by the numerous available methods are influenced by such different factors that it seemed desirable to conduct pot experiments with kinds of plants which are markedly favored by liming, in order that a basis might be available for forming a judgment of the practical value of the chemical methods.

The samples were taken to represent the permanent plats 23, 25, 27 and 29 of the Rhode Island station. The treatment which these plats had received to the close of 1914, and other information concerning them, is given in Bulletin 160 of the station. Subsequently, miscellaneous crops were planted in 1915 and 1916. In the spring of 1915, plat 23, called the unlimed ammonium sulfate plat, for the first time was given an application of hydrated lime equivalent to 500 pounds of calcium oxide per acre; plat 25, 1500 pounds; and plat 29, 1000 pounds. Plat 27 has never been limed.

Liberal amounts of nitrogen, phosphorous and potassium have been added annually to each plat alike since 1893. To plats 23 and 25 the nitrogen was supplied in sulfate of ammonia and to 27 and 29 in nitrate of soda. The physiological acidity and basicity of the respective forms of nitrogen have been shown plainly in the crop results.

The soil was air-dried, sifted through about 2-mm. holes and, after removing samples for Mr. Veitch, 12 pounds placed in each 8-inch Wagner pot. So-called c. p. calcium oxide was hydrated with an equivalent amount of water and passed through a 200-mesh sieve in preparation for liming certain pots. Special attention was paid to bringing about as complete a reaction as possible between the soil and the lime with the hope of obtaining the maximum effect of the lime for the immediate crop. The applications of lime and

[1] Contribution No. 241 from the Agricultural Experiment Station of the Rhode Island State College. Presented at the Convention of the Association of Official Agricultural Chemists in 1916.

fertilizers were mixed thoroughly with the moist soil at intervals during two weeks before planting early Egyptian beet seeds on July 28, 1916; the crop was harvested November 6, 1916. The basic nutrients were proved to be optimum by additional pots receiving calcium in sulfate, and extra potassium, nitrogen and phosphorus. The results are presented in table 1.

TABLE 1

Relation of the lime requirements to the effect of lime on beets

CaO added per acre	Weight of beet roots, average from two pots				CaO requirements per acre after the growth of beets			
	Unlimed $(NH_4)_2SO_4$ plat	Limed $(NH_4)_2SO_4$ plat	Unlimed $NaNO_3$ plat	Limed $NaNO_3$ plat	Unlimed $(NH_4)_2SO_4$ plat	Limed $(NH_4)_2SO_4$ plat	Unlimed $NaNO_3$ plat	Limed $NaNO_3$ plat
pounds	*gm.*	*gm.*	*gm.*	*gm.*	*pounds*	*pounds*	*pounds*	*pounds*
None	0	211	37	386	7,800	5,800	7,300	5,100
378				352				5,100
757		300		418		5,600		5,000
1,135	83	311	308	434	6,900	5,500	6,000	5,000
1,513	210	364	313		6,700	5,100	5,400	
1,892	248		365		6,500		5,300	
2,270	318				5,000			
3,780	351				5,000			
Before the growth of beets:								
Ammonia method					6,900	5,200	5,500	3,700
Veitch method					8,700	5,800	8,100	4,800

The ammonia method was used in general in determining the lime requirements given in this paper. It may be found in Soil Science, v. 6, p. 405–511.

In November, 1916, a new lot of soil was taken from the same plats and a second experiment conducted in a manner similar to the first, except that instead of beets, cos lettuce was planted February 3, 1917. The crop was harvested April 26, 1917. The results given are in table 2.

TABLE 2

Relation of the lime requirements to the effect of lime on lettuce

CaO added per acre	Weight of green lettuce, average from two pots				CaO requirements per acre after the growth of lettuce			
	Unlimed $(NH_4)_2SO_4$ plat	Limed $(NH_4)_2SO_4$ plat	Unlimed $NaNO_3$ plat	Limed $NaNO_3$ plat	Unlimed $(NH_4)_2SO_4$ plat	Limed $(NH_4)_2SO_4$ plat	Unlimed $NaNO_3$ plat	Limed $NaNO_3$ plat
pounds	*gm.*	*gm.*	*gm.*	*gm.*	*pounds*	*pounds*	*pounds*	*pounds*
None	0	36	12	143	8,300	6,200	6,400	4,800
500				160				
1,000		168	147	181		5,600	5,800	4,700
1,500	74	163	180	162	7,300	5,300	5,500	
2,000	157	182	181		6,400	5,000	5,200	
3,000	174				6,200			
4,000	201			142	5,800			2,800

By reference to tables 1 and 2 it may be seen in general that after maximum crops had been attained by liming, the soil still showed a lime requirement of about 5000 pounds; in fact, the lettuce, although a crop which is very sensitive to "soil acidity," was depressed in growth when an application equivalent to 4000 pounds of calcium oxide was made to the limed nitrate of soda soil, and the subsequent lime requirements thereby reduced to 2800. A carbon-dioxide determination made in this soil showed that not more than about 10 per cent of the added lime remained in carbonate at the end of the experiment.

At the bottom of table 1 are the lime requirements of the soil before it was used in the beet experiment. It may be seen that the limed nitrate of soda soil had a lime requirement of 3700 by the ammonia method and yet not much advantage was derived from liming. When about this amount was added for lettuce there was less crop than where smaller amounts were used. And yet it may be seen that the Veitch method yielded even higher results than the ammonia method.

Although some very interesting correlations could be shown between the lime requirements as determined by the plant and by the chemist, it is desired rather to refer sufficiently to certain results obtained at the Rhode Island station, so that the chemist will not make the mistake of associating lime requirement too closely with soil acidity, and of giving too little attention to the varying characteristics of different kinds of plants as related to other deleterious factors. Only a very brief reference to the indications afforded by our work should be made in this connection. The details will be given elsewhere, with such modifications as the terse statements here made for the sake of brevity may require.[2]

As illustrating the importance of considering the plant in its relation to the soil, let us consider rye and barley seedlings. They are affected alike by acidity, and yet very differently by acid soils. Under field conditions where rye is not benefited by liming, barley is increased many fold. The aqueous extract of the soil affects the two seedlings similarly as the soil, as do also the ignited residues when brought into solution. Aluminum is a prominent constituent of the residue. When to nutrient solutions this element is added as aluminum sulfate, for example, in comparison with equivalent amounts of sulfuric acid, the two seedlings are depressed alike by the acid, whereas the aluminum sulfate, although it causes a depression in the growth of the barley similar to that with the acid, has comparatively little effect on the rye.

Emphasis has been laid heretofore on the toxicity of aluminum salts because of their hydrolysis and consequent effect on the acidity. The extent of the hydrolysis was sufficient to produce in the nutrient solution only about one-fourth the hydrogen-ion concentration that was produced by the equivalent

[2] Hartwell, Burt L., and Pember, F. R. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *In* Soil Sci., v. 6, p. 259–278.

amount of acid. The greater part of the depression in the growth of barley therefore was attributed largely to the aluminum itself rather than to the acidity produced by the hydrolysis of its salts, whereas the aluminum is much less toxic to rye.

Accordingly, the elimination of the effect of the aluminum in acid soils seems likely to prove of more importance than the neutralization of the acidity, and attention should be given to methods of determining active aluminum while we are also developing those for soil acidity. Perhaps the principal materials for rendering aluminum inactive in the soil will prove to be lime and acid phosphate. Especially the latter substance has been shown to decrease the amount of aluminum soluble in carbonated water and dilute acetic acid. Its application in large amounts to a soil in which crops greatly benefited by liming are to be grown, may enable those crops to make a satisfactory growth. For example, in the lettuce experiment described previously some of the soil from the unlimed ammonium sulfate plat was thoroughly mixed in a moist condition with large amounts of acid phosphate in addition to the phosphorus added in the basal manures for nutrient purposes. The mixture was allowed to react about two weeks previous to planting the lettuce. The crop could make no growth on the check soil, but 74 gm. per pot were produced with acid phosphate at the rate of 14 tons per acre, and 272 gm. when used at the extraordinary rate of 28 tons. By reference to table 2 it may be seen that the next largest yield in the entire experiment was 201 gm., which was where lime equivalent to 2 tons of calcium oxide per acre was applied to the same soil. Nevertheless, at the close of the experiment the lime requirements were found to be 12,000 pounds of calcium oxide per acre where the larger amount of acid phosphate was used, 9000 where half the amount was used, 8300 for the check soil where only the basal nutrients were added, and 5800 where the 2 tons of calcium oxide were applied.

A substance which increased the high lime requirement at least for a time so changed a very acid soil that an application of lime was unnecessary.

THE AMMONIA-FIXING CAPACITY OF CALCIUM SULFATE

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INTRODUCTION

That much nitrogen is lost from manure by the volatilization of ammonium compounds is generally conceded. The extent to which this loss can be prevented by the use of chemicals as preservatives is not well established. The use of calcium sulfate for this purpose is a very old practice. Concerning its efficiency as an ammonia fixer there is little agreement among investigators. There is also a difference of opinion among those who believe it will conserve ammonia as to the manner in which it does it.

Some agriculturists have entertained the view that calcium sulfate has the ability to hold ammonia gas by physical adsorption. Some have contended that the retention of the ammonia may be due to a process of chemical absorption, resulting in the formation of a double sulfate. Far the greater number have considered that the benefit from using calcium sulfate as a preserver depends upon its ability to transform volatile ammonium carbonate into the more stable ammonium sulfate by the process of double decomposition.

According to the results of Haskins (8) physical adsorption plays a very small part, if any, in the fixation of ammonia.

The work of Bell and Taber (3) makes the second of these suppositions appear untenable, since all the conditions necessary to the formation of $CaSO_4$, $(NH_4)_2SO_4.x(H_2O)$ would not obtain in a manure heap.

The third assumption, that of double decomposition, is based on rather reasonable grounds both theoretical and experimental. However, the degree to which the reaction takes place in decomposing organic matter is questionable. Since the reaction is a reversible one, and since calcium sulfate is comparatively insoluble, it would appear doubtful whether under the normal moisture conditions in manure, very much ammonium sulfate is formed before a state of equilibrium is reached. Even after being formed, if some of the moisture is lost in any way, the reverse reaction very probably predominates and produces again some of the unstable ammonium carbonate. It is well known that when $(NH_4)_2SO_4$ and $CaCO_3$ are mixed together intimately in the dry state, or at least with only hygroscopic moisture present, a distinct odor of ammonia is noticeable. Obviously this is due to the formation of volatile $(NH_4)_2CO_3$. It is also easy to believe that changes of temperature and other physical conditions may have a great influence on the direction in which

the reaction proceeds. Reactions with gypsum in a pile of fermenting material are no doubt somewhat complicated. Sulfates, being oxidizing substances, are likely to be reduced to sulfides by the action of the carbonaceous matter present. The sulfides formed may interact with H_2CO_3 to form carbonates.

HISTORY OF INVESTIGATIONS

Considerable investigational work has been carried on to determine the power of calcium sulfate to fix ammonia. In most cases gypsum in variable quantities has been mixed with manure, allowed to stand for a time, and by analysis of the manure for nitrogen before and after the investigation a conclusion has been reached as to the value of the gypsum as a preservative. The fact that much of the nitrogen is given off in a free state, as shown by Russell and Richards (17) as well as by other investigators (6) has been little considered. A few experiments of a modified type have been carried on.

Although many of these investigations have only a general bearing on the problem under consideration, it seems desirable to review briefly some of the results that have been obtained and some opinions and conclusions that have been reached.

The earlier writers on agriculture have said much regarding the ability of calcium sulfate to fix ammonia. Boussingault (4) states that M. Shattermann, who was a large horse keeper of Alsace during the first half of the nineteenth century, managed large heaps of manure wery well and used either sulfate of iron or gypsum as preservatives.

Liebig (9) believed that the beneficial effects which were observed where calcium sulfate had been strewn on a meadow were due to the ability of the salt to fix the ammonia of the atmosphere by the formation of ammonium sulfate. He describes an experiment which was made with growing beans and peas on a garden plot, which had been well manured and strewed with gypsum. Before beginning the experiment it was found by analysis that both soil and gypsum were quite free from carbonates; but on examination of some of the salt taken from the surface of the ground after several weeks, it was discovered that the greater part of the gypsum had been converted into the carbonate of lime and that the soil to a depth of six inches effervesced strongly on the addition of acid. This corresponds to the results obtained by Spatzier and reported by Storer (18). Lemmerman and Fresenius (11) record similar results.

From an extended investigation covering 6 years of time, on how to reduce the loss of nitrogen from manure, Müntz and Girard (13) concluded that the use of chemical agents is unsatisfactory and that, in the case of calcium sulfate, so much must be used to attain the desired result as to make the practice uneconomical.

Burri, Herfeldt and Stutzer (5) inoculated nutrient solutions with manure bacteria, some with bacteria from old decomposing liquid manure and some with bacteria from fresh liquid. Various manure preservatives were added to

the cultures and results noted. One of their conclusions was that "gypsum has no value as a preservative. It does not combine with the carbonate of ammonia already present and does not prevent the further formation of this substance even when used in large quantities."

Aeby, Dorsch, Matz and Wagner (1) found that in case of complete humification and long storage the denitrifying action in manure is lessened by being reinforced with gypsum, but concluded that as a fixer of ammonia it is of little use.

Aso and Nishimuro (2), in studies on the comparative value of superphosphate of calcium, gypsum and kainit as manure preservatives found that gypsum and kainit are much inferior to superphosphate as fixers of ammonia.

Lindsey (10) reviewed the results of German investigators and found the consensus of opinion to be against the use of chemical preservatives such as gypsum, on the ground that the nitrogen saved is quite out of proportion to the expense involved.

Pichard (15) compared the action of calcium sulfate and ferrous sulfate in conserving soil nitrogen. The substances were mixed with artificial soils of known composition and placed in enameled earthenware pots. The gypsum, which was added at the rate of 5 gm. per kgm., was found to be more favorable to nitrification and a more effective conserver of nitrogen than ferrous sulfate.

At the Ohio Agricultural Experiment Station (21) work has been carried out to determine the effect on crops of the use of reinforced manure. The manure was applied at the rate of 8 tons per acre, in a 3-year rotation of corn, wheat and clover, and the reinforcing materials were used at the rate of 40 pounds to the ton of manure. In the trial with gypsum-treated yard manure the increase over untreated manure was 7.37 bushels of corn and 473 pounds of stover, 4.07 bushels of wheat and 452 pounds of straw and 581 pounds of hay. In the trial with stall manure the increase was 3.18 bushels of corn and 211 pounds of stover, 2.35 bushels of wheat and 201 pounds of straw, but with a loss of 134 pounds of hay. These are the averages for 16 crops of corn, 16 of wheat and 13 of hay. The results of 5 years' analyses of treated and untreated manure used in these tests, before and after 3 months' exposure to winter weather, showed a nitrogen loss of 29.6 per cent when reinforced with gypsum and 35.5 per cent when no preservative was used (20).

Dietzell (7) placed samples of manure in 12-liter flasks carrying tubes through which a current of air could be drawn. Gypsum and other preservatives were mixed with different samples. The temperature at which the flasks were kept ranged from 10.5° to 25°C. Analysis was made of the manure at the beginning and at the end of the experiment to determine the nitrogen content. The ammonia which passed out through outlet tubes was collected by means of standardized sulfuric acid. Gypsum and kainit proved to be the most efficient preservatives tried in preventing the loss of nitrogen. It was also found that, if the air is excluded from manure, no preservative is needed to

conserve the nitrogen. This corresponds with the recent conclusion of Russell and Richards (17) that the nitrogen loss from manure is least when stored under anaerobic conditions.

Meyer (14) investigated the effect of various preservatives on 100-cc. portions of liquid manure. He found that from 92.8 to 95.9 per cent of the nitrogen of the liquid was in the form of ammonium carbonate, and that it was practically all lost when allowed to stand for some time exposed to the air. Approximately 50 per cent of the nitrogen was saved when gypsum was used as a preservative.

Severin (19) sterilized manure and inoculated it with cultures of manure bacteria. By using 4 per cent of gypsum as a preserver he succeeded in reducing the loss of ammonia by approximately one-half. He also noted a faster decomposition of the manure treated with gypsum than of that not so treated. The greatest loss observed, however, when no $CaSO_4$ was used was only 0.0739 gm. from 265 gm. of manure in 90 days. Not only was it concluded that gypsum is an efficient preserver of ammonia but also that the value of acid phosphate for this purpose is due to the calcium sulfate it contains.

Palmer (16) carried out an investigation similar in many respects to that of Severin but the quantities of manure were greater and his purpose was only to determine the efficiency of various chemicals as preservatives of ammonia in manure. Fifty-four pounds of horse manure or 150 pounds of cow manure were placed in galvanized iron cans. The chemical preservatives were added at the rate of 80 pounds to the ton of manure. In the case where $CaSO_4$ was used 32.53 mgm. of nitrogen was lost from 100 pounds of cow manure in 62 days as compared to 52.47 mgm. when no preservative was added. In the trial with horse manure 32.08 mgm. was lost in 44 days compared to 73.18 mgm. when no $CaSO_4$ was used.

The majority of investigators seem inclined to the view that gypsum possesses some virtue as a nitrogen conserver. The way in which this is done and the quantitative effect are still unsettled problems.

NATURE AND PURPOSE OF THIS EXPERIMENT

Most, if not all, of the attempts to determine the capacity of $CaSO_4$ to fix ammonia in manure have involved the use of decomposing organic matter and urine as the source of the ammonia. Since many and complicated biochemical reactions take place in a mass of fermenting manure, and since the quantity of ammonia and ammonium carbonate actually formed is not known, it seems desirable to investigate the ammonia-fixing ability of $CaSO_4$ independent of decomposing organic matter, using as the source of the ammonia a definite quantity of pure ammonium carbonate. In this way many troublesome factors are eliminated. The number of possible reactions also is limited and the actual capacity of $CaSO_4$ to hold the ammonia of ammonium carbonate by the process of double decomposition of these salts can, it would seem, be more definitely estimated.

EXPERIMENTAL PROCEDURE

Four trains of bottles were arranged as shown in figure 1. The size of the bottles marked A was 2.5 liters. In each of these was placed 250 gm. of filter paper, which had been previously pulped fine and dried, as a substitute for the organic matter of the manure. With the filter paper added to each of two of these bottles, 150 gm. of pure $CaSO_4$ was intimately mixed. The material in the bottles was compacted and 750 cc. of a solution containing 39.30 gm. of $(NH_4)_2CO_3$ was added to each. This amount of $(NH_4)_2CO_3$ would be equivalent to 9.645 gm. of nitrogen or 19.29 pounds to the ton of material. The water content was made approximately 75 per cent. The $CaSO_4$ was added at the rate of 300 pounds to the ton of material. The temperature was varied

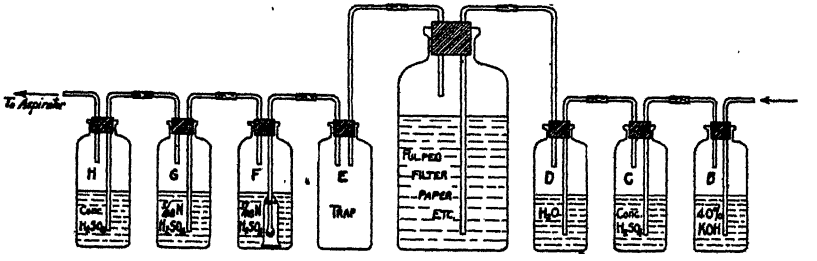

FIG. 1. DIAGRAM SHOWING ARRANGEMENT OF APPARATUS USED IN EXPERIMENT

from time to time, as will be seen by reference to tables 1 and 2. Temperatures higher than room temperature were obtained by placing the bottles marked A inside a box lined with asbestos and heated with an electric lamp. Lamps of different candle power were used to obtain the different degrees of heat. By this arrangement it was possible to control the temperature to within a comparatively narrow range. The ammonia which escaped was collected in standard acid solutions in bottles F and G.

The bottles marked C and H contained concentrated H_2SO_4 to absorb the ammonia of the air. Bottle B was partly filled with a 40 per cent solution of KOH to remove the CO_2 from the incoming air and bottle D contained distilled water to insure the entrance of sufficiently moist air to maintain the water content of A. Air was aspirated through the apparatus from time to time for 10-minute periods, the actual number of aspirations being shown in table 1.

DISCUSSION OF RESULTS

It will be noted by an examination of the results of the experiment as summarized in tables 1, 2, and 3 that quite a large amount of nitrogen was given off during the period of the test by bottles containing no $CaSO_4$. In those treated with $CaSO_4$ the quantity given off was 79.7 per cent less. This was a much larger saving of nitrogen than was found by either Severin (19) or

TABLE 1

The effect of $CaSO_4$ *in preventing nitrogen loss*

NUMBER OF DAYS	NUMBER OF ASPIRATIONS	RANGE OF TEMPERATURE	NITROGEN LOSS	
			Without $CaSO_4$	With $CaSO_4$
		°C.	*gm.*	*gm.*
9	4	20°–25°	A. 0.0240	A. 0.0055
			B. 0.0180	B. 0.0030
15	8	20°–25°	A. 0.0460	A. 0.0005
			B. 0.0410	B. 0.0015
11	6	20°–30°	A. 0.0754	A. 0.0074
			B. 0.0764	B. 0.0079
20	19	20°–30°*	A. 0.2510	A. 0.0240
			B. 0.2560	B. 0.0200
12	12	45°–55°	A. 0.1870	A. 0.0250
			B. 0.2620	B. 0.0270
10	10	50°–59°	A. 0.3940	A. 0.0310
			B. 0.3090	B. 0.0270
9	15	65°–70°	A. 0.7420	A. 0.1150
			B. 0.6260	B. 0.1420
5	10	65°–90°§	A. 0.7520	A. 0.1120
			B. 0.7045	B. 0.1050
4	7	80°–85°	A. 0.8467	A. 0.2565
			B. 0.8485	B. 0.2510
			A. 0.0105†	A. 0.0090†
			B. 0.0100†	B. 0.0095†
			A. 2.6150‡	A. 0.4900‡
			B. 2.1500‡	B. 0.6200‡
95	91		A. 5.9436	A. 1.0749
			B. 5.3014	B. 1.2139
			Average, 5.6225	Average, 1.1419

* Temperature for one day of period was over 30°C.

† Ammonia collected in bottles marked G during period of test.

‡ Ammonia absorbed by water condensed in trap bottles.

§ Temperature was 65°–70° during 4 days of period and raised to 90° one day.

Palmer (16), the former having found that $CaSO_4$ reduced the loss approximately 50 per cent and the latter 38 per cent in the case of cow manure and 56.2 per cent in the case of horse manure. It will be recalled, however, that the quantity of NH_3 given off in their experiments was quite small, due partly to the fact that they depended on the decomposition of manure for the ammonia supply and partly, no doubt, to a lower temperature.

It may be thought that the temperature to which the mixture of filter paper, $(NH_4)_2CO_3$, etc., was raised in the latter part of this investigation was too high to be analogous to the conditions in a manure heap. While it is true that ordinarily the temperature of manure is not so high, it was found by Russell and Richards (17) that the temperature of fermenting horse manure under cover may rise to 70–80°C. It seemed desirable, therefore, to note the results

at high temperature in order to determine the efficiency of $CaSO_4$ as a preservative under such conditions.

The quantity of nitrogen given off by the two bottles of a pair is quite different in some cases, especially at the higher temperatures. This can be largely accounted for by the difficulty experienced in keeping the source of heat at the same distance from the bottles of the mixture.

A comparatively large quantity of the nitrogen evolved was absorbed as ammonia by water condensed in the trap bottles. No condensation of mois-

TABLE 2

Daily nitrogen loss with and without $CaSO_4$

PERIOD	TEMPERATURE	WITHOUT $CaSO_4$	WITH $CaSO_4$
	°C.	*per cent*	*per cent*
First	20°–25°	0.024	0.006
Second	20°–25°	0.030	0.001
Third	20°–30°	0.072	0.007
Fourth	20°–30°	0.132	0.011
Fifth	45°–55°	0.197	0.023
Sixth	50°–59°	0.365	0.030
Seventh	65°–70°	0.854	0.148
Eighth	65°–90°	1.511	0.225
Ninth	80°–85°	2.197	0.659

Note: The ammonia absorbed in trap bottles and that neutralized in bottles marked G is not included in these calculations.

TABLE 3

Total nitrogen loss with and without $CaSO_4$

PERIOD	TEMPERATURE	WITHOUT $CaSO_4$	WITH $CaSO_4$
		per cent	*per cent*
During first 55 days	Room temperature	4.08	0.33
During last 40 days	45°C. and above	54.21	11.50
Total loss 95 days		58.29	11.84

ture was noticeable in these bottles until the beginning of the fifth period, when the temperature of the mixture was raised above room temperature. In compiling table 3, therefore, it was thought well to consider that all ammonia collected in the trap bottles was given off during the last forty days.

A consideration of the results as shown by the tables would seem to leave little doubt that $CaSO_4$ will hold some ammonia and that it does it by interacting with $(NH_4)_2CO_3$ to form the non-volatile compound $(NH_4)_2SO_4$. This last assumption is based on the probability of other reactions being prevented by the conditions under which the experiment was performed. It is very probable that many reactions take place in a manure heap, subsequent to the formation of $(NH_4)_2SO_4$ that would result in an ammonia loss. One of these is the possi-

ble reduction of sulfates to sulfides by the carbonaceous matter present. We know, too, that the reaction $(NH_4)_2CO_3 + CaSO_4 = (NH_4)_2SO_4 + CaCO_3$ is reversible and that it is largely a question of mass action as to which direction the reaction will go.

When a large amount of water is present the formation of $(NH_4)_2SO_4$ takes place until equilibrium is reached. When the percentage of water is lowered the reverse reaction no doubt predominates. When the state known as "fire-fanged" is reached in a manure-heap, under which condition a comparatively small percentage of water is present, it would seem reasonable to believe that most of the ammonia of the $(NH_4)_2SO_4$, that had been formed, would be changed to the carbonate and be lost. A plentiful supply of moisture, therefore, seems necessary, if $CaSO_4$ is to be of real service as a manure preservative. Such a condition obtained in this experiment and also in those of Severin and Palmer. It is perhaps the reason also that Meyer (20) found gypsum able to hold practically half the ammonia of liquid manure. It would seem desirable to investigate the amount of ammonia given off during a few weeks of time from a mixture of filter paper, $(NH_4)_2SO_4$ and $CaCO_3$ at comparatively high heat and low moisture content.

An examination of the table will show that $CaSO_4$ apparently loses some of its ability to hold ammonia as the temperature is raised. The relative amount held at high temperature is not as great as at low.

SUMMARY AND CONCLUSIONS

It appears to be unquestionable that $CaSO_4$ will prevent to a certain degree the loss of ammonia from a mixture containing volatile $(NH_4)_2CO_3$ and it is quite probable that the loss prevention is due to the formation of non-volatile $(NH_4)_2 SO_4$ by the double decomposition of $(NH_4)_2CO_3$ and $CaSO_4$, for no other means is thought to be possible under the conditions of this experiment.

The ammonia-fixing capacity of $CaSO_4$ is apparently dependent on the temperature. The quantity of ammonia given off by the $(NH_4)_2CO_3$ increases as the temperature rises and the percentage of it held by the $CaSO_4$ decreases.

A treatment of 300 pounds of $CaSO_4$ to the ton of manure would, no doubt, effect a pronounced ammonia fixation, if the water content were comparatively high. Whether subsequent reactions that probably take place in a manure heap would prevent its remaining fixed and whether the practice of reinforcing manure with gypsum is economical are questions beyond the scope of this investigation.

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PARALLEL FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATE IN SOIL

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The biological significance of carbon-dioxide formation in the soil has long been recognized and its measurement under varying conditions has attracted the attention of many students of soil biology. Potter and Snyder (2) have recently reviewed the literature relative to this subject. Similarly, the vast amount of literature relative to ammonia and nitrate formation in soils attests the importance attached by students of soil biology, to a better understanding of the factors controlling these very important processes.[1] It has long been recognized that to a large extent the processes through which carbon dioxide, ammonia and nitrates are formed in soil are biological in nature. The fact that the formation of any one of these compounds may take place independently of the others, has resulted in each process being studied more or less independently of the others. There are few instances on record where the parallel formation of carbon dioxide, ammonia and nitrates in a soil has been studied.

When the experiments herein described were undertaken it was proposed to carry out rather extensive investigations along this line. Other work, however, necessitated an indefinite delay, so that the publication of the results of a few of the more suggestive experiments that have been completed seems desirable at the present time.

METHODS

Any one who has attempted to make quantitative measurements of carbon dioxide evolved continuously over a considerable length of time realizes the difficulties to be overcome in making such measurements accurately. In soils, the difficulties are many times multiplied.

Since these experiments were carried out (late in 1916 and early in 1917) several methods, perhaps more accurate for measuring carbon dioxide evolved from soils, have been suggested by other investigators. However, the methods employed gave, as a rule, consistent results, and duplicate determinations agreed as well as could be expected.

The question of whether the air used to sweep the carbon dioxide out of the soil should be drawn over or through the soil has often been raised. The writer is of the opinion that so long as the production of carbon dioxide and

[1] The results presented in this paper on ammonia and nitrate formation are not such as will necessitate a general consideration of previous work along similar lines.

the consumption of oxygen are not greatly stimulated by the addition of easily oxidized organic matter, drawing air over the soil will remove carbon dioxide and keep up a sufficient supply of oxygen to maintain aerobic conditions. However, if the column of soil is of considerable depth and the evolution of carbon dioxide and the consumption of oxygen are as rapid as in these experiments, it is questionable whether aerobic conditions can be maintained unless the air is drawn through the soil. It is also questionable, if the air is simply drawn over the soil, whether the removal of carbon dioxide will be sufficiently rapid for the records to indicate the rate of formation. For these reasons the air was drawn through the soil in all experiments here reported.

Figure 1 represents a complete unit of the apparatus used and needs little explanation. The soil was placed in a a calcium chloride cylinder, a perforated porcelain plate preventing the soil from falling into the foot of the cylinder. The cylinder was then connected through the side opening, by means of glass tubing, to a small wide-mouth bottle containing a 10 per cent solution of sodium hydroxide. Into the stopper of this bottle was also placed a straight-form calcium chloride tube 1 inch in diameter, the larger bore being 10 and the smaller bore 4 inches long. This tube was filled nearly full with solid glass beads. The bottle contained such a quantity of 10 per cent solution of sodium hydroxide that when suction was applied the liquid drawn into the tube would cover about three-fourths of the beads. The tube containing beads was in turn connected with another bottle and tube similar in every respect except that it contained a dilute acid. The acid should be of such a strength that an even moisture content will be maintained in the cylinder of soil to which the bottle containing acid is connected.

Six such units were usually connected in a series. The air before entering the first cylinder was passed through sodium hydroxide and acid wash towers similar to those used for catching carbon dioxide and ammonia. The whole was connected with a water pump. Between the last cylinder and the pump was placed a trap sufficient in size to release the partial vacuum in case of a lowered water pressure. If there is danger of irregularity in suction a third wash tower, containing a solution of sodium hydroxide, so arranged that the current of air passes through in the reverse order, can be introduced between the acid wash tower and the succeeding cylinder of soil. This would increase the necessary suction only slightly and would prevent any carbon dioxide from a cylinder of soil being caught by a wash tower to the rear. With wash towers arranged as indicated no liquid can be drawn backward in case of accidental reversal of the air current.

With six such units connected to one pump the resistance to the current of air and the total volume of air under partial pressure are such that an even flow of air bubbles was not obtained but rather an intermittent passage of bubbles through the various wash towers. This has the advantage of bringing about frequent changes of liquid in the wash towers.

The quantity of carbon dioxide caught was measured by thoroughly washing the beads with carbon-dioxide-free water and determining the carbon dioxide present by the double titration method. Ammonia caught in the acid wash towers can be determined by distillation or by the aeration method. In no instance in the experiments herein reported was the quantity of ammonia given off sufficient to be taken into consideration. This was true even though the quantity of nitrogen present as ammonia was often more than 30 mgm. in 100 gm. of soil.

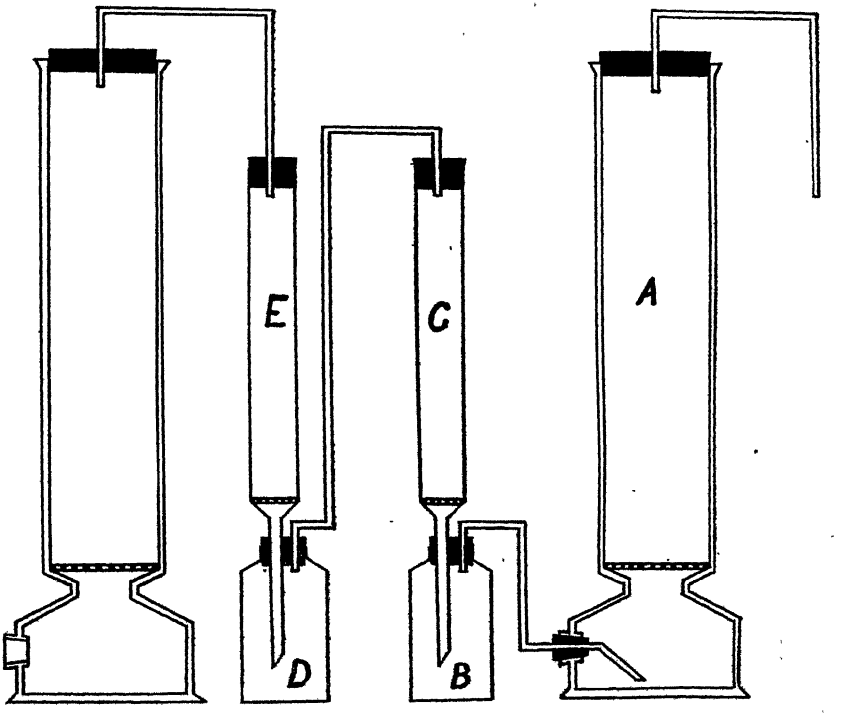

FIG. 1. A UNIT OF APPARATUS USED IN EXPERIMENTS

a, Calcium chloride cylinder containing soil; *b*, bottle containing 10 per cent NaOH; *c*, calcium chloride tube containing glass beads; *d*, bottle containing dilute acid; *e*, calcium chloride tube containing glass beads.

A sufficient quantity of soil was removed from the top of the cylinder at each analysis to make duplicate quantitative ammonia and nitrate-nitrogen determinations. For the former, analyses were made by the aeration method. Nitrate nitrogen was determined by extracting with water and using either the colorimetric or aluminum reduction method. The carbon-dioxide content of the soil was not taken into consideration.

Carbon dioxide is reported in milligrams given off between the various analyses, a correction being made upon a basis of the initial quantity of soil. Am-

monia and nitrates are reported in milligrams of nitrogen per total quantity of initial soil. The curves for carbon dioxide are constructed by adding the total quantity given off prior to any particular analysis. In most instances cylinders of soil were run in duplicate or triplicate, and the curves in such instances are based upon averages. It occasionally happened that an analysis, particularly of carbon dioxide, was evidently incorrect, possibly because of a leak in the system. Such records were discarded.

The same soil, a silt loam of approximately the following composition was used in all experiments: coarse grades of sand 0.9 per cent; fine grades of sand 8.4 per cent; silt 71.8 per cent; and clay 17.8 per cent. The water-holding capacity of this soil in loose condition is approximately 54 cc. per 100 gm. of soil.

EXPERIMENTAL DATA

Experiment A, recorded in table 1 and figure 2, was run to determine how closely the duplicate carbon-dioxide determinations would check. Six 1000-gm. samples of soil containing 20 gm. of water per 100 gm. of soil were weighed

TABLE 1

Experiment A. CO_2 production in soil with (samples 3–6) and without (samples 1–2) 1 per cent cottonseed meal

SAMPLE	CO_2 EVOLVED DURING THE PERIODS INDICATED IN DAYS										
	1	2	3	4	5	6–7	8–9	10–11	12–14	15–17	Total
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	165	149	198	195	97	156		231*		183†	1,374
2	136	129	182	129	100	136	178			172	1,164
3	1,726	2,380	1,945	1,202	791	885	671	414	348	347	10,709
4	1,928	2,614	1,969	1,364	830	1,113	816	458	416	394	11,902
5	1,829	2,436	1,951	1,257	813	974	664	414	348	332	11,018
6	1,809	2,446	1,839	1,169	818	948	704	481	387	321	10,922

* Eighth to eleventh day.

† Twelfth to seventeenth day.

out. To two samples no further additions were made. These were placed in cylinders 1 and 2. To the remaining four 1 per cent cottonseed meal was added and thoroughly mixed. These samples were placed in cylinders 3 to 6. Air was drawn through the soil continuously for 17 days except when analyses were being made. For the first few days carbon-dioxide analyses were made daily at the same time of day; afterwards the period between analyses increased as the quantity of carbon dioxide evolved decreased. No analyses of ammonia and nitrate nitrogen were made in this experiment.

An examination of the carbon dioxide curves, figure 2, will show that the evolution of carbon dioxide was very uniform, giving exceptionally regular curves. Since all subsequent experiments received an addition of cottonseed meal or dried blood, the curves in this experiment for soil with no addition need not

be considered further. The agreement between duplicates is also considered good. For example, the probable error for the analyses on the fifth day is ±1.00 and for the total carbon dioxide evolved during the 17 days ±1.58.

There was a very rapid formation of carbon dioxide during the first few days; the maximum apparently occurring during the second 24 hours. This may possibly be due to the fact that the day the experiment was begun cannot be regarded as a full 24 hours, since it required at least half a work-day to set the

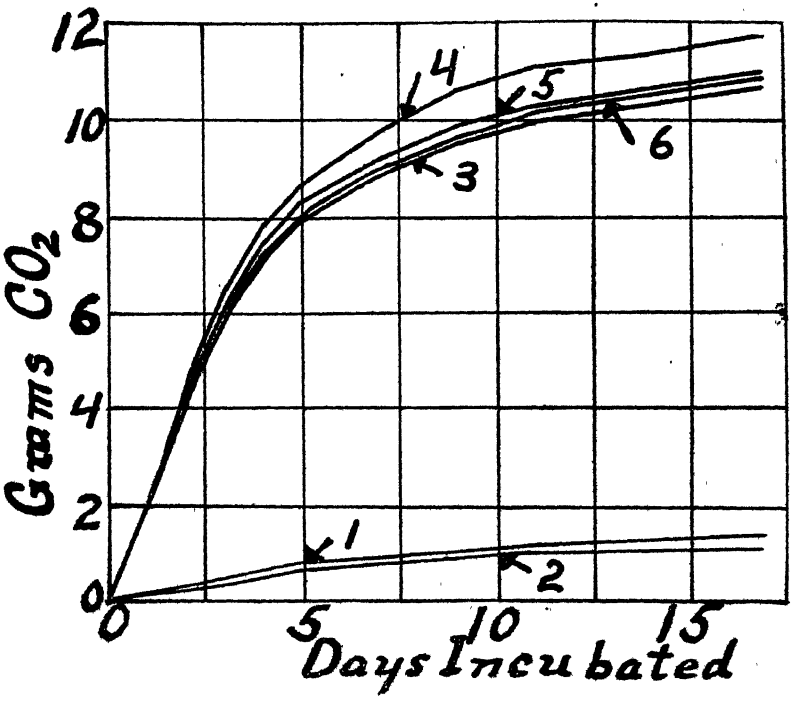

FIG. 2. CARBON DIOXIDE FORMATION IN EXPERIMENT A

Samples 1 and 2: No cottonseed meal added. Samples 3 to 6: 1 per cent cottonseed meal added. Moisture content 20 cc. per 100 gm. soil. Aerated continuously.

experiment up. By the end of 3 days 50 per cent and by the end of 7 days 75 per cent of the total carbon dioxide given off during the course of the experiment had been evolved. After the first week the quantity given off decreased very rapidly, the curves soon assuming the form of straight lines. For convenience the carbon dioxide curves of this experiment will be spoken of as normal or typical carbon-dioxide curves.

EFFECT OF VARYING THE MOISTURE CONTENT

Experiment B, recorded in table 2, and figure 3, was performed to study the effect of varying the moisture content upon parallel carbon dioxide, ammonia, and nitrate formation in soil. One thousand grams of soil containing 1 per

TABLE 2

Experiment B. Formation of CO_2, ammonia and nitrates in soil containing varying quantities of moisture; 1 per cent cottonseed meal added

DAYS	MILLIGRAMS OF CO_2 AND NITROGEN AS NH_3 AND NO_3 PER 1000 GM. OF SOIL														
	No. 1; 12 cc. H_2O			No. 2; 22 cc. H_2O			No. 3; 32 cc. H_2O			No. 4; 42 cc. H_2O			No. 5; ? cc. H_2O		
	NO_2	NH_3	NO_3	CO_2	NH_3	NO_2	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	1,958	19.5	0	2,161	50.5	0	2,585	37.5	0	2,295	34.2	0	2,018	27.7	0
2	2,260	48.9	0	3,200	152.4	0	3,254	153.2	0	2,651	93.0	0	2,744	121.4	0
3	1,674	111.1	0	1,626	241.8	0	1,846	222.2	0	1,668*	233.7	0	1,528	245.1	0
4	1,087	141.1	0	854	281.1	Trace	958	281.7	Trace	1,087	258.2	0	1,100	264.7	Trace
5	962	197.7	Trace	481	297.4	5.4	784	317.0	6.8	770	267.9	Trace	839	323.5	4.5
6	264	194.4	4.5	513	300.6	6.8	287	295.7	11.4	360	276.2	3.4	396	277.8	5.6
7– 8	436	201.0	6.1	220	310.5	11.3	251	312.1	23.5	353	289.2	6.8	353	294.1	18.0
9–10	309	214.0	9.0	277	302.3	14.9	157	277.8	27.0	152	305.6	16.9	237	217.0	33.8
11–12	220	227.0	11.3	55		18.0	90	326.8	45.0	165	274.0	24.6	137	294.0	33.9
13–15	128	262.4	13.5	73	325.2	23.5	73	303.9	54.1	153	269.5	54.1	124	317.0	64.9
16–22		212.9	15.0		247.9	27.0		255.6	67.5		218.9	79.2		209.4	91.8
23–28	284	159.4	27.2	78	219.0	38.7	96	190.0	93.4	154		108.0	124	130.4	122.4

E:

cent cottonseed meal and the following quantities of water were placed in the corresponding cylinders: no. 1, 12 cc.; no. 2, 22 cc.; no. 3, 32 cc., no. 4, 42 cc.; and no. 5, 52 cc. When the air current was started a very small quantity of water was drawn out of no. 4 and a rather large quantity out of no. 5. In other words, 42 cc. per 100 gm. of soil was all the water this soil would hold and at the same time permit the passage of a current of air. Moisture determinations at the end of the experiment showed no change in the content of no. 1 and 4; no. 2 and 3 showed slight increases; while no. 5, showed a decided decrease. No curves are plotted for no. 5.

The production of carbon dioxide was remarkably uniform, giving typical curves. There were very slight differences in carbon dioxide formation compared with the wide differences in moisture content. The maximum carbon-

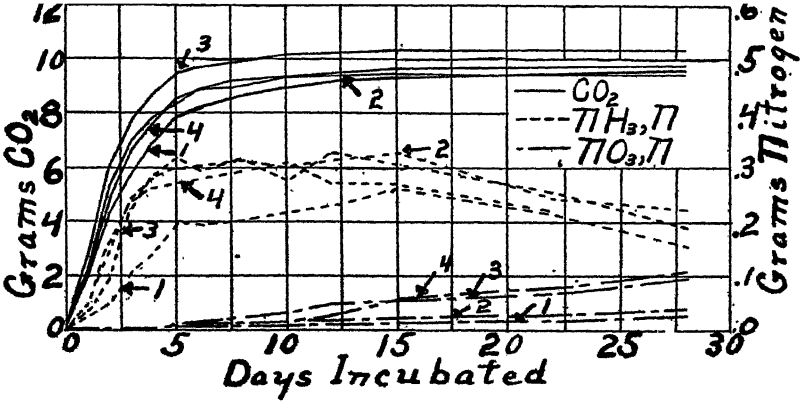

FIG. 3. FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATES IN EXPERIMENT B

Soil contained 1 per cent cottonseed meal and was aerated continuously. Moisture content: No. 1, 12 cc.; No. 2, 22 cc.; No. 3, 32 cc.; No. 4, 42 cc. per 100 gm. soil.

dioxide production took place during the second 24 hours. By the end of a week approximately 90 per cent of the carbon dioxide evolved during the four weeks the experiment was running had been given off.

The ammonia nitrogen curves for those samples containing more than 12 cc. of water per 100 gm. of soil follow very closely the carbon dioxide curves until nitrification begins. After nitrification became active the ammonia curves fell as the nitrate curves rose. The ammonia curve for the sample containing 12 cc. of water per 100 gm. of soil falls much below the others. This would indicate that the quantity of moisture necessary for favorable ammonia production is higher than for favorable carbon dioxide production.

The nitrate nitrogen when plotted gave practically straight lines. Nitrates did not begin to accumulate until about the fifth day, and for some unknown reason nitrification in all samples was very slow for this soil. The quantities of nitrate nitrogen present increased as the moisture content was raised up to the maximum moisture that the soil would retain.

TABLE 3

Experiment C. Formation of CO_2, ammonia and nitrates in soil containing varying quantities of water; 1 per cent cottonseed meal and 0.5 per cent calcium carbonate added

DAYS	MILLIGRAMS OF CO_2 AND NITROGEN AS NH_3 AND NO_3 PER 1000 GM. OF SOIL																	
	11 cc. H_2O per 100 gm.						26 cc. H_2O per 100 gm.						41 cc. H_2O per 100 gm.					
	No. 1			No. 2			No. 3			No. 4			No. 5			No. 6		
	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	643	26.1	6.8	665	14.7	6.8	1,776	17.1	Trace	1,870	13.0	Trace	2,090	11.4	0	2,020	11.4	0
2	940	12.2	3.4	928		Trace	1,650	66.8	0	1,568	51.3	0	1,650	35.6	0	1,627	31.0	0
3	776	16.3	3.4	898	13.0	Trace	2,039	163.0	0	1,944	153.2	0	1,827	117.4	0	1,790	109.2	0
4	800	13.0	3.0	875	19.2	Trace	1,533	205.4	3.4	1,690	197.2	3.4	1,546	145.1	0	1,780	151.6	0
5	695	27.7	4.5	695	27.7	3.0	1,052	244.5	4.5	1,052	238.0	4.5	1,038	200.5	Trace	1,025	168.0	Trace
6	675	44.0	4.5	785	40.7	3.0	815	269.7	7.5	726	267.1	6.8	915	211.1	Trace	910	179.3	Trace
7– 8	605	57.1	7.5	668	60.3	6.8	636	283.6	16.2	652	224.9	16.2	793	246.0	7.7	859	267.0	8.1
9–11	761	78.5	12.2	778	88.0	10.6	787	270.6	38.5	745	260.8	35.0	1,007	214.3	19.4	1,015	208.0	16.0
12–15		83.2	3.4	541	92.2	3.4	568	241.0	32.7	541	234.7	34.0	725	175.8	85.0	815	161.0	90.0
16–24		135.0	4.5	720	107.9	3.5	870	252.2	21.4	820	233.2	21.4	860	220.2	13.7		210.7	12.0
25–39		170.8	6.2	1,056	153.2	6.0	1,050	172.8	210.0		146.4	210.0	1,221	16.3	420.0		16.3	420.0

There is some irregularity in the quantity of ammonia recorded from the fifth to the fifteenth day due no doubt to the inability to secure an absolutely representative sample of soil when only the surface soil is taken. So far, no method has been devised for overcoming this objection without at the same time upsetting other experimental conditions.

Experiment C, recorded in table 3 and figure 4, also has to do with the influence of varying the moisture content. One thousand grams of soil containing 1 per cent cottonseed meal and 0.5 per cent calcium carbonate were placed in each of six cylinders. No. 1 and 2 contained sufficient moisture to bring the content up to 11 cc. per 100 gm. of soil. The moisture content of no. 3 and 4, and that of no. 5 and 6 were brought, respectively, to 26 and 41 cc. per 100 gm. of soil. The experiment was continued for 39 days.

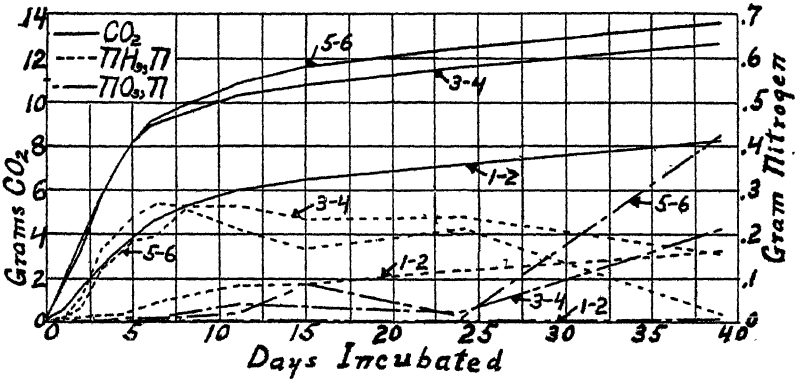

FIG. 4. FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATE IN EXPERIMENT C

Soil contained 1 per cent cottonseed meal and 0.5 per cent calcium carbonate and was aerated continuously. Moisture content: No. 1 and 2, 11 cc.; No. 3 and 4, 26 cc.; No. 5 and 6, 41 cc. per 100 gm.

Samples 3 to 6 gave the normal carbon dioxide and ammonia curves. Samples 1 and 2, however, evidently did not contain sufficient moisture for maximum bacterial activity. This is evident from the formation of carbon dioxide, ammonia and nitrate. The moisture in this instance is only slightly less than that present in sample 1, experiment B. There is a large difference, however, in the rate of carbon dioxide and ammonia formation. This would indicate that the minimum moisture content for active carbon dioxide and ammonia formation for this soil is near 12 cc. per 100 gm. of soil. For some unknown reason nitrification was depressed somewhere between the fifteenth and twenty-fourth day analyses. Aside from this irregularity the curves are regarded as satisfactory. This depression in nitrification caused a second rise in the ammonia curves. The moisture content of samples 1 and 2 was not sufficient for nitrate accumulation to take place. Apparently the only effect of the calcium carbonate was to increase slightly the total carbon dioxide evolved.

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Experiment D. Formation of CO_2, ammonia and nitrates in soil variously aerated; 1 per cent cottonseed meal and 22 per cent water added

DAYS	MILLIGRAM OF CO_2 AND NITROGEN AS NH_3 AND NO_3 PER 2000 GM. OF SOIL														
	Aerated continuously						Aerated ½ hour daily								
	No. 1			No. 2			No. 4			No. 5			No. 6		
	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	4,840	267	Trace	4,950	256	Trace	1,496	24	0	1,496	20	0	1,474	16	0
2	3,768	436	8.0	3,776	466	7.0	1,010	30	0	976	46	0	1,118	34	0
2	2,304	476	16.3	2,460	500	15.0	1,424	56	0	1,450	87	0	1,305	88	0
2	1,448	492	19.0	1,482	540	19.0	1,542	216	0	1,694	264	0	1,644	276	0
2	1,010	524	28.0	1,046	552	27.0	1,734	304	0	2,234	360	0	2,272	352	0
4	1,566	560	42.0	1,777	566	42.0	4,576	424	0	4,506	424	9.0	4,540	440	9.0
6	880	580	74.0	1,160	580	74.0	4,560	472	34.0	2,900	472	62.0	3,440	436	62.0
9	722	564	90.0	892	544	94.0	2,100	468	108.0	1,460	412	134.0	1,482	464	125.0
12	1,008	504	169.0	1,255	484	169.0	1,320	292	300.0	1,172	272	338.0	1,205	256	319.0

EFFECT OF VARYING AERATION

The following experiments were carried out to ascertain the effect of varying the rate of aeration upon parallel formation of carbon dioxide, ammonia and nitrate in the same soil. In experiment D (table 4 and figure 5) six cylinders of 2000 gm. of soil containing 1 per cent cottonseed meal and 22 cc. of water per 100 gm. of soil were prepared. Through no. 1, 2, and 3, a current of air was drawn continuously. Through no. 4, 5 and 6 the air was drawn ½ hour daily just before the analyses were made. The experiment was continued for 41 days. The results from cylinder 3 were not regarded as satisfactory and are not recorded.

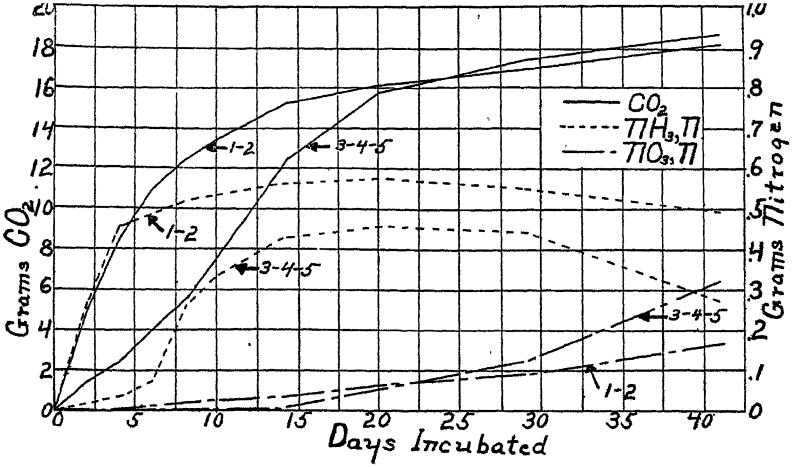

FIG. 5. FORMATION OF CARBON DIOXIDE AMMONIA AND NITRATES IN EXPERIMENT D

Soil contained 1 per cent cottonseed meal and 22 cc. water per 100 gm. No. 1 and 2 aerated continuously, No. 3 to 5 aerated ½ hour daily.

The samples aerated continuously gave the characteristic carbon dioxide and ammonia curves. The carbon-dioxide curve for samples aerated ½ hour daily lagged behind and equalled the normal curve only after 3 weeks. The ammonia curve for these samples was depressed even more markedly than the carbon dioxide curve and failed to reach the normal curve during the course of the experiment. If the carbon dioxide curve were the only measure of bacterial activity available it might be assumed that aerating ½ hour daily failed effectively to remove carbon dioxide. The ammonia determinations, however, were not dependent upon volatilization, and since it was depressed more than the carbon dioxide formation we are justified in assuming that the carbon dioxide curve actually represents carbon-dioxide formation.

The more or less anaerobic conditions existing in the poorly aerated samples for the first two weeks did not exist after that time, else no accumulation of nitrate nitrogen could have taken place. The vigorous oxidation of the easily oxidized organic matter probably utilized all available oxygen. As such

TABLE 5

Experiment E. Formation of CO_2 and ammonia in soil variously aerated; 1 per cent cottonseed meal and 22 per cent water added

DAYS	MILLIGRAMS OF CO_2 AND NITROGEN AS NH_3 PER 1750 GM. OF SOIL							
	Aerated continuously				Aerated ½ hour daily			
	No. 1		No. 2		No. 3		No. 4	
	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3	CO_2	NH_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	4,588	77	4,048	80	1,628	10	1,452	10
2	4,796	388	4,356	395	1,276	17	1,012	21
2	2,090	427	1,947	462	1,001	38	913	66
2	1,276	469	1,364	476	1,309	161	847	210
4	1,034	455	1,210	448	2,354	304	1,980	285

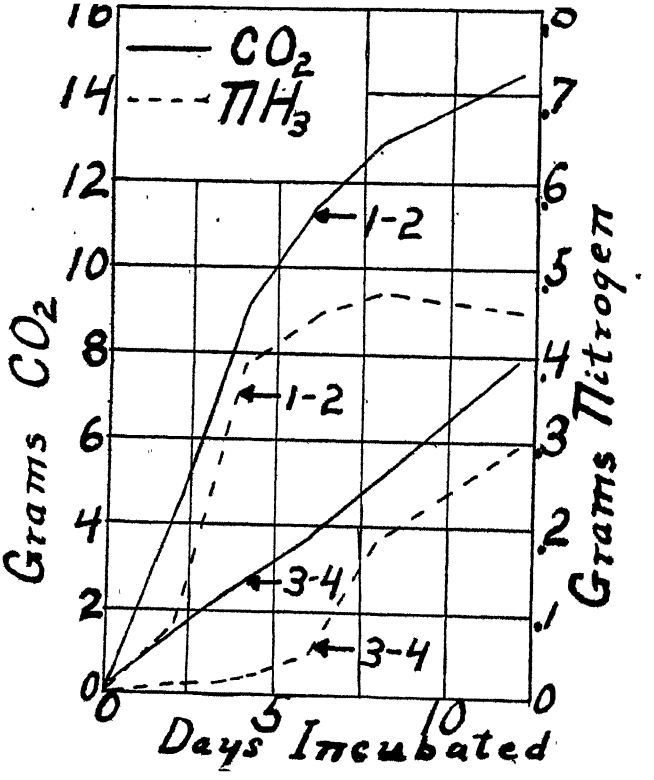

FIG. 6. FORMATION OF CARBON DIOXIDE AND AMMONIA IN EXPERIMENT E

Soil contained 1 per cent cottonseed meal and 22 cc. water per 100 gm. No. 1 and 2 aerated continuously, No. 3 and 4 aerated ½ hour daily.

organic matter rapidly became less a smaller quantity of oxygen was needed for this purpose, and finally reached after two weeks, a point where sufficient oxygen was available for both the oxidation of organic matter and for active nitrification. After nitrification began in the less highly aerated samples, it was much more vigorous than in the better aerated cylinders. This is in accord with results previously published by the writer (1) in which it was shown that the accumulation of nitrate nitrogen took place more rapidly in the bottom of cylinders of soil than in the top, provided aeration was not entirely eliminated by a high moisture content.

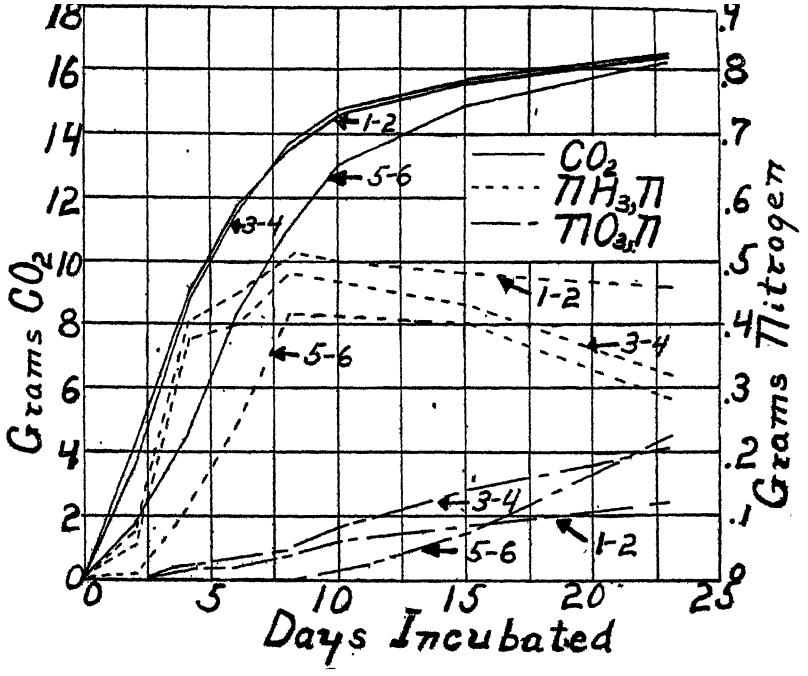

FIG. 7. FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATES IN EXPERIMENT F

Soil contained 1 per cent cottonseed meal and 21 cc. water per 100 gm. No. 1 and 2 aerated abundantly and continuously. No. 3 and 4 aerated slowly though continuously. No. 5 and 6 aerated 8 a.m. to 6 p.m.

Experiment E is a duplicate of experiment D except that 1750 gm. of soil were used instead of 2000. A comparison of the data secured from this experiment, table 5 and figure 6, with that of experiment D will show that one is practically a duplication of the other. This experiment was continued for only 12 days and the quantities of nitrate nitrogen were so small that they are not recorded.

In experiment F, recorded in table 6 and figure 7, 2000 gm. of soil containing 1 per cent cottonseed meal and 21 cc. of water per 100 gm. of soil, were

TABLE 6

Experiment F. Formation of CO_2, ammonia and nitrates in soil variously aerated; 1 per cent cottonseed meal and 21 per cent water added

DAYS	MILLIGRAMS OF CO_2 AND NITROGEN AS NH_3 AND NO_3 PER 2000 GM. OF SOIL																	
	Aerated strongly, continuously						Aerated slow, continuously						Aerated 8 a.m. to 6 p.m.					
	No. 1			No. 2			No. 3			No. 4			No. 5			No. 6		
	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	4,290	80	0	4,048	84	Trace	3,850	56	Trace	3,960	60	Trace	1,826	10	0	1,716	10	0
2	5,160	404	13	4,446	392	12	5,160	372	17	4,632	372	17	2,870	102	0	2,756	100	0
2	2,982	456	22	2,640	444	22	3,128	428	30	2,884	368	34	3,788	248	Trace	3,920	248	0
2	1,656	504	38	1,824	516	43	1,954	476	43	1,805	484	54	2,692	424	0	2,744	408	0
2	1,138	504	67	1,088	496	59	1,016	460	81	958	468	85	1,948	428	17	1,988	408	11
5	1,012	472	81	914	492	85	1,100	424	141	934	440	135	1,830	404	76	1,945	404	72
8	878	476	122	980	444	122	890	346	208	764	298	208	1,414	286	230	1,433	286	216

placed in each of 6 cylinders. Through no. 1 and 2 a strong current of air was drawn all the time. Through no. 3 and 4, a continuous current of air was drawn but the volume was reduced as low as was possible to still be kept passing. The former was at least ten times as strong as the latter. Through no. 5 and 6 the current of air was drawn from 8 a.m. to 6 p.m., being run ½ hour before analysis. The experiment was continued for 23 days.

The carbon dioxide and ammonia curves for samples 1 to 4 are again typical. Both the carbon dioxide and ammonia curves for no. 5 and 6 lag somewhat behind, indicating that aeration only during the work day was not sufficient to maintain optimum conditions for aerobic bacterial activity. Again, nitrate accumulation in the less highly aerated cylinders was more rapid when once established than in the other cylinders. The accumulation of nitrate nitrogen in this experiment is in agreement with experiment E.

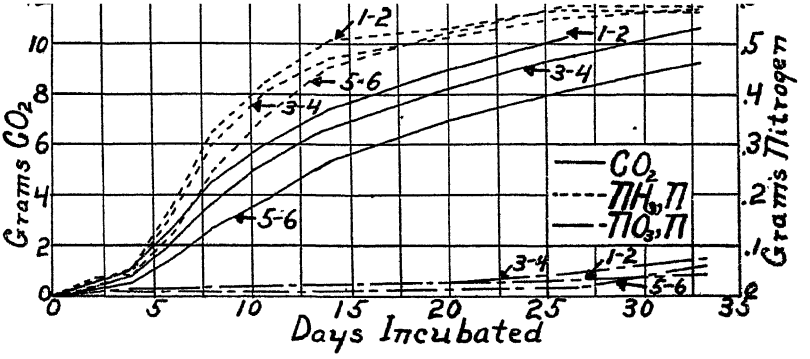

FIG. 8. FORMATION OF CARBON DIOXIDE, AMMONIA AND NITRATES IN EXPERIMENT G

Soil contained 1 per cent dried blood and 22 cc. water per 100 gm. No. 1 and 2 aerated continuously. No. 3 and 4 aerated 8 a.m. to 6 p.m. No. 5 and 6 aerated 1 hour daily.

One thousand grams of soil containing 1 per cent dried blood and 22 cc. of water per 100 gm. of soil were used in experiment C. Through no. 1 and 2 the current of air was kept continuous, through no. 3 and 4 from 8 a.m. to 6 p.m., and through no. 5 and 6 one hour daily just before being analysed. The results are recorded in table 7 and figure 8.

The difference in the character of the organic material added modifies decidedly the character of the curves for carbon dioxide and ammonia. The nitrogen content of this dried blood was approximately twice that of the cottonseed meal. The relative position of the carbon dioxide and ammonia curves is reversed, the latter being higher than the former. This is apparently due not to an increased ammonia formation but to a depressed carbon dioxide formation.

During the first four days under normal conditions where cottonseed meal was added, approximately 50 per cent of the carbon dioxide and ammonia formed

TABLE

Experiment G. Formation of CO_2, ammonia and nitrates in soil variously aerated; 1 per cent dried blood and 22 per cent water added

DAYS	MILLIGRAMS OF CO_2 AND NITROGEN AS NH_3 AND NO_3 PER 2000 GM. OF SOIL																	
	Aerated continuously						Aerated 8 a.m. to 6 p.m.						Aerated 1 hour daily					
	No. 1			No. 2			No. 3			No. 4			No. 5			No. 6		
	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3	CO_2	NH_3	NO_3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	479	19.5	11.9	402	13.7	11.2	391	24.4	11.9	193	25.4	10.6	248	34.2	11.9	83	27.4	11.2
2	648	60.6	11.9	745	56.7	11.9	608	48.9	11.2	486	54.7	11.2	421	46.9	11.9	292	46.9	11.2
2	1,454	175.9	11.9	1,625	180.1	11.9	1,412	168.1	11.9	1,223	156.4	10.6	1,018	132.9	8.1	802	121.2	10.6
2	1,812	318.7	15.0	1,883	344.1	15.0	1,766	297.2	16.4	1,304	297.2	15.0	1,268	244.4	0	1,187	238.5	10.6
3	1,723	436.0	18.0	1,694	430.1	17.0	1,761	404.7	16.4	1,721	414.5	14.0	1,482	367.5	0	1,068	365.6	10.4
3	1,333	480.9	18.0	1,345	533.7	18.0	1,223	455.5	16.4	1,437	488.8	16.7	1,530	449.7	0	1,407	461.4	10.4
6	1,457	561.1	23.8	1,507	498.5	23.8	1,496	510.3	23.8	1,611	518.1	23.0	1,760	520.0	6.3	1,450	523.9	11.9
6		588.5	29.2	1,068	553.3	27.7	1,068	545.4	40.0	1,457	561.1	36.0	1,256	563.0	18.0	1,019	588.5	18.0
7					568.9	40.0	1,104	563.0	79.9	1,232	574.8	59.9	1,272	570.9	72.0	1,181		45.0

during the course of the experiments, was recovered. From dried blood, only small quantities were recovered at the end of 4 days. After the fourth day analyses the curves for ammonia were very similar to those in previous experiments. The formation of carbon dioxide, however, was still comparatively slow, the most rapid evolution taking place between the sixth and eighth day analyses. After this the curves tend gradually toward straight lines, but with a more decided tendency upward than shown by carbon dioxide curves for the latter stages of the experiments where cottonseed meal was added. These various facts would indicate that the organic carbon of dried blood is not nearly so easily oxidized by soil organisms as is the carbon of cottonseed meal. Neither was the nitrogen of dried blood liberated as ammonia in this experiment as rapidly or as completely as was that of cottonseed meal. The experimental data available are not sufficient for us to conclude that this would hold true for other soils, or for other samples of dried blood and cottonseed meal in the same soil.

The effect of variation in aeration is evident both on the carbon dioxide and ammonia curves. The depressions due to decreased aeration are not, however, of the same nature or degree as in those experiments where cottonseed meal was added to the soil.

The nitrate nitrogen accumulated very slowly in all samples, but toward the end of the experiment nitrification appeared to be inversely proportional to aeration.

DISCUSSION AND SUMMARY

In studying the several sets of data, and curves constructed therefrom, several rather striking facts are to be noted. In the first place there is a remarkable similarity and agreement between the several carbon dioxide and ammonia curves when constructed from data secured under anything like similar conditions. If conditions are favorable for bacterial activity and cottonseed meal is added, there is at first a very rapid formation of carbon dioxide and ammonia, the maximum being reached during the second 24 hours. After this the formation of both carbon dioxide and ammonia decreased rapidly, the curves soon tending toward straight lines. When dried blood was added to the soil the curves for both carbon dioxide and ammonia were different. Formation during the first 4 days was very slow, the maximum being reached between the sixth and eighth day analyses. The formation of carbon dioxide from dried blood was never so rapid as from cottonseed meal, but was apparently distributed over a longer period of time.

Insufficient moisture retards both carbon dioxide and ammonia formation, the latter much more markedly. The minimum moisture for active carbon-dioxide production is lower than for active ammonia production. For the soil used in these experiments 12 cc. of water per 100 gm. soil was near the minimum for vigorous carbon-dioxide production, and increasing the moisture

beyond this point had little effect upon the formation of carbon dioxide. A moisture content below this results in a lowered carbon-dioxide production. The moisture necessary for optimum ammonia formation is higher than that for carbon dioxide.

Insufficient aeration will also cause a depression or marked delay in carbon dioxide and ammonia production. The data here recorded are insufficient as a basis for a statement as to the oxygen necessarily available for maximum aerobic biological activity. In no instance where the current of air was not continuous was the production of carbon dioxide and ammonia equally as rapid as where it was. In the case of carbon dioxide it appears to be simply a delayed production, the total quantity soon reaching that produced where aeration was abundant. The total ammonia produced under limited aeration did not, in most instances, reach the production where aeration was abundant. So long as the current of air was continuous, increasing the volume apparently had no effect.

Where moisture and aeration are both favorable the ammonia production follows very closely the evolution of carbon dioxide. Unfavorable conditions, however, have a more detrimental effect upon the formation of ammonia than upon the formation of carbon dioxide.

Carbon dioxide production from dried blood is not nearly so rapid as it is from cottonseed meal. Neither is the curve similar. There is a relatively greater ammonia production per unit of carbon dioxide produced from dried blood than from cottonseed meal. This is probably due to the higher nitrogen content. Per unit of nitrogen, that contained in cottonseed meal is liberated much more rapidly than that in dried blood. This may possibly be due to the particular sample of dried blood though it has usually been the writer's experience than when equal quantities of nitrogen are added to soil in the two forms that in cottonseed meal is ammonified more rapidly. The low carbon dioxide production would seem to indicate that the rapidly oxidizable carbon was perhaps so limited that sufficient energy was not available for breaking up the nitrogen combinations. If ammonia is simply a by-product, as sometimes regarded, and sufficient quantities of easily oxidized carbon were present in the dried blood, then it would seem we should get proportionately higher ammonia liberated where the higher per cent of nitrogen was present.

Nitrate production in all experiments was for some unknown reason exceptionaly low for this particular soil. As a rule there was no accumulation during the first few days. In fact the small quantity of nitrate nitrogen initially present (left out of consideration in records) usually disappeared completely before the first analysis. Where aeration was insufficient the beginning of accumulation was delayed still more.

Where the moisture content was varied, nitrate accumulation was directly proportional to moisture content, the maximum not being reached until the maximum moisture that would be retained was reached. Where aeration

was varied insufficient aeration retarded the initial accumulation, but after nitrification became active in all samples the accumulation was inversely proportional to aeration.

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THE MOVEMENT OF MOISTURE IN SOIL BY CAPILLARITY

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The individuals of a mass of spherical soil particles of uniform size will come in contact at a maximum of twelve points, and if moisture be present it will adhere as a thin film about the surface with ring-shaped water wedges about the points of contact. From a consideration of the geometrical configuration, it may be seen that changes in curvature of the air-water surface depend upon changes in the moisture content. It is well known that dry soil in contact with moist soil will absorb a part of the moisture, and the above explanation is generally accepted by soil workers.

For the case of horizontal capillary movement, hydrostatic pressure does not enter and the gradient of the curvature pressure is a measure of the kinematical force acting on the water which is free to move. The thin hygroscopic film is held (1) to be in a solid phase or in loose chemical combination and in this discussion may perhaps be regarded as a part of the solid soil particle. Where the pore space is completely filled with water the air-water surface disappears and movement depends upon the hydrostatic pressure gradient. Slichter (2) has observed that the hydrostatic pressure in this case satisfies Laplace's equation and he has made some extended theoretical mathematical calculations for the flow of under-ground water under pressure, which have found fair experimental verification. The only assumption underlying his theory is that the velocity is proportional to the pressure gradient, and by substituting for the velocity components in the equation of continuity for incompressible fluids these simple functions of the pressure, he arrives immediately at Laplace's equation.

The essential distinction between the case considered by Slichter and the case of capillary flow would seem to be that the density (i.e., the mass of moisture per unit volume in the soil) is dependent upon the space coördinates and also upon the time; also that the pressure is a function of the density. It is to be noted that the relative motion of rigid soil particle and moisture surrounding it is somewhat analagous to the case of the motion of a particle falling through a viscous fluid, and, on the basis of Stokes' law for this case, the assumption is made that the velocity is proportional to the pressure gradient, but the pressure is proportional to the curvature and the curvature is a determinate function of the density for the idealized case under consideration. The air-water surface configuration in a typical soil is, however,

only approximately similar to the ideal case and therefore a rigorous determination of this function has not been attempted. By reference to figure 1 it will be observed that the water wedge is enclosed by the surface of revolution generated by revolving arcs *ab*, *bc*, and *ca* about the line of centers of the spheres. The radii of curvature of the principal sections are oppositely directed and vanish together, but the one will approach the radius of the soil grain as a maximum while the other becomes infinite. The following relation has been assumed:

$$-r_1 = \frac{ar_2}{br_2 + c} \tag{1}$$

where r_1 and r_2 are the radii of curvature and a, b, and c are undetermined constants.

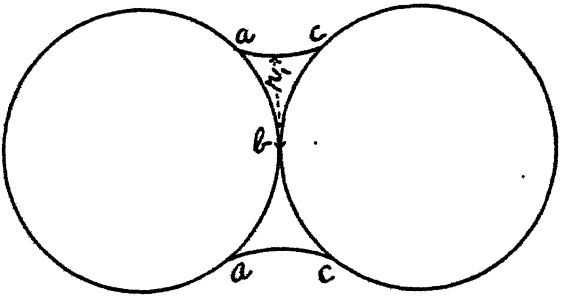

FIG. 1. OUTLINE OF WATER WEDGE BETWEEN TWO SOIL PARTICLES

The pressure under a curved surface may be expressed:

$$p = 2T(1/r_1 - 1/r_2) \tag{2}$$

where T is written for the surface tension and p for the pressure. The volume of the water wedge, which is proportional to the density, is also a function of r_1 and we have assumed that it is proportional to r_1^3,

$$\rho = k_1 r_1^3 \tag{3}$$

where ρ is the density, k_1 an undetermined constant. From equations (1), (2), and (3), we may arrive at once at the relation:

$$dp/dx = k_2/\rho^{\frac{1}{3}} d\rho/dx \tag{4}$$

k_2 being a constant involving a, b, c, T and k_1.

Stokes' law may be expressed:

$$v = k_3/\eta \, dp/dx \tag{5}$$

where k_3 involves the radius of the soil particle and η is the coefficient of viscosity of the liquid ($\frac{dp}{dx}$ substituted for the gravitational acceleration constant).

The equation of continuity for one-dimensional flow may be written:

$$\frac{\partial \rho}{\partial t} = -\frac{\partial}{\partial x}(\rho v) \tag{6}$$

and a combination of (4), (5), and (6) gives,

$$\frac{\partial \rho}{\partial t} = -\frac{k_3}{\eta}\frac{\partial}{\partial x}\left(\frac{\partial \rho}{\partial x}\cdot\frac{1}{\rho^3}\right) \tag{7}$$

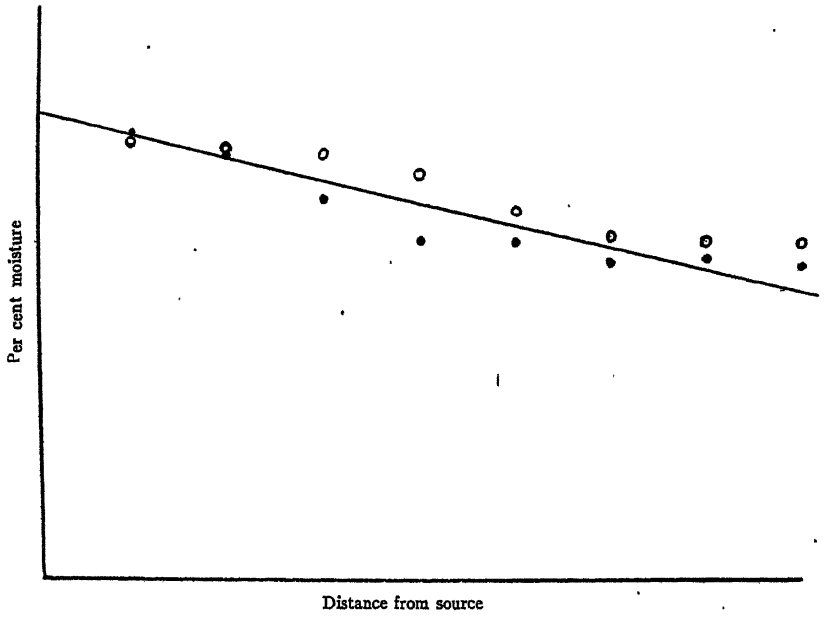

FIG. 2. DIAGRAM ILLUSTRATING WIDSTOE'S DATA

Small circles represent data from irrigated farms, black dots data from dry farms.

as the differential equation for capillary motion. For the case of a steady state, this becomes,

$$\rho\frac{\partial^2 \rho}{\partial x_2} = \tfrac{1}{3}\left(\frac{\partial \rho}{\partial x}\right)^2 \tag{8}$$

and the solution

$$\rho = (Ax + B)^{3/2} \tag{9}$$

where A and B are integration constants.

A number of experimental cases have been considered. Widtsoe (3) has made extended investigations in soil moisture and finds the winter precipitation distributed in early spring as illustrated in figure 2. The curve is plotted from equation (9) with assumed values for A and B. The small circles rep-

resent Widtsoe's data from irrigated farms and the black dots data from dry farms.

In the laboratory at the Utah Experiment Station several preliminary observations have been made as to the moisture distribution at the steady state. The moisture was allowed to penetrate by capillarity into horizontal tubes of about 4 cm. diameter containing fine sand. The moisture was

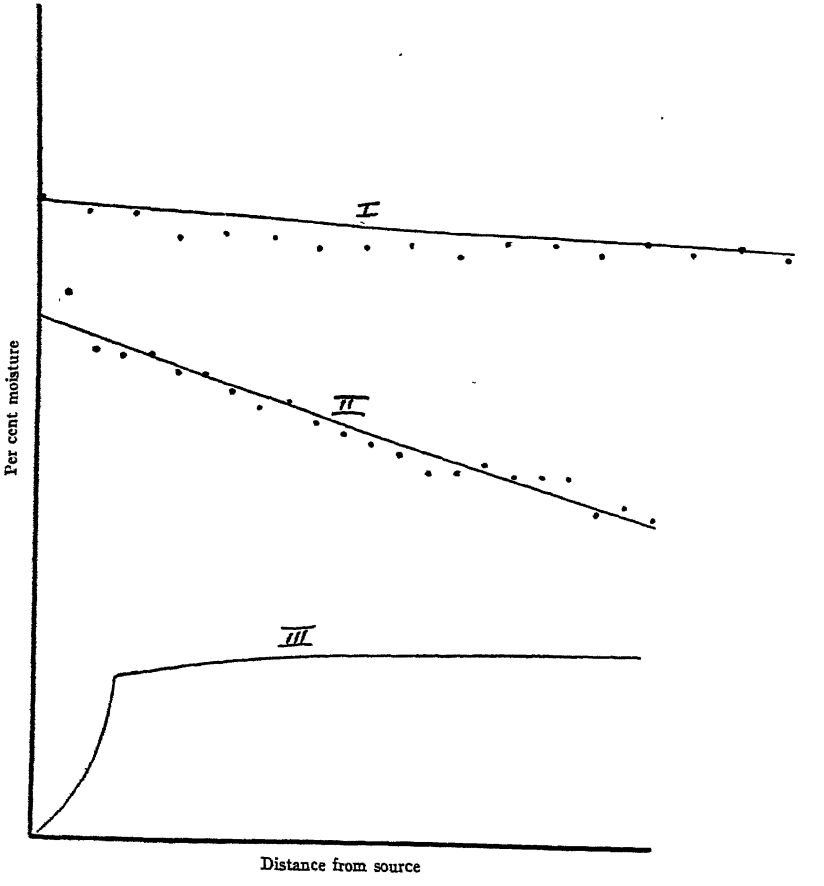

FIG. 3. CURVES SHOWING LABORATORY DATA AND MOMENT OF MOISTURE AS IT VARIES WITH THE TIME

evaporated from the remote end. The tubes, about 6 feet in length, were mounted in such a way as to rotate about an axis perpendicular to the tube and a record of the changing moment due to the moisture as it crept forward was kept as a guide to the degree to which a steady state was approached. In figure 3, curve III, is plotted the moment of the moisture as it varied with the time, the ordinate in this case being the moment and the abscissa the time.

Curve II of figure 3 was calculated from equation (9) with assumed values for A and B. The dots represent the mean of a series of three experimental determinations. In curve I are shown the results of a single observation made with a different soil and with a slightly different method, the curve again in this case being calculated and the dots representing experimental values.

The assumptions which have been made in the theoretical considerations above may affect the magnitude of the exponent in equation (9) but the experimental data would seem to indicate that equation (9) is not far from correct. As will be noted, the experimental errors are rather large, owing to the fact that slight irregularities in the manner of packing the soil and other attendant experimental difficulties tend to modify results obtained.

In conclusion, I wish to acknowledge the able assistance rendered by Mr. Scott Ewing in obtaining the experimental results in the laboratory and the coöperative encouragement of Dr. F. L. West, the head of the Physics Department.

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CAPILLARY MOISTURE-HOLDING CAPACITY

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It has been pointed out that the moisture-holding capacity as defined by Hilgard (7) does not correspond with the amount actually found in the field after irrigation or heavy rain. Israelsen (8, p. 22) gives ratios of the amount found by a method similar to the Hilgard method to the amount found in the field after irrigation ranging from 1.32 to 2.30; and Alway (1, p. 410) gives ratios of 1.5 to 2.

Briggs (4, 5) explained the movement of capillary water on the basis of well known surface tension phenomena and his view seems to be quite generally accepted. Widtsoe (10) discusses soil moisture from the standpoint of film thickness, indicating several stages in the mobility of water over the soil grains. Bouyoucos (3) has given evidence that the hygroscopic film may be in a solid phase and it is maintained that there may be a somewhat unstable chemical combination of a part of this moisture with the soil. It is believed, however, that water in excess of that designated by Widtsoe as the lento-capillarity point may respond to the capillary forces. In fact, from the mechanical point of view, water of hydration and "solid" water alone may be excluded, but soil water in such condition is only a small part of the total quantity present in ordinary moist soils.

On the basis of these facts, we are confronted with the inevitable deduction that a state of equilibrium in soil moisture, exclusive of the hygroscopic moisture, would demand a curvature gradient, and consequently a moisture gradient, of such a magnitude as to supply a force equal to the force due to gravity, assuming that adhesive forces are negligible beyond the lento-capillarity point. There is abundant evidence that capillary movement is comparatively slow and static equilibrium is the exception rather than the rule. It is therefore to be expected that a decided difference may exist between the moisture capacity of a soil as defined by Hilgard and the average amount found in the region of root activity immediately after heavy irrigation or at any subsequent time, although an accidental agreement may be found at some stage during the process of adjustment to equilibrium. Alway (1, p. 415) reports the ratio of the water content to the hygroscopic coefficient in the first and second 3-inch sections of two series of soil tubes, the first of which were allowed to stand five days and the second from 66 to 110 days after "irrigation," protected from surface evaporation. His results are

quoted in table 1. It would no doubt be admitted that final equilibrium had not been attained although with a low moisture content the movement may be very slow.

In a somewhat similar experiment, King (9) found that the process of readjustment continued in some cases for 2½ years, but in every case the final moisture per cent was found to increase with increasing depth. In

TABLE 1

Extent to which equilibrium had been attained by the different soils at the end of 5 days, as shown by comparing the ratios of water content to hygroscopic coefficient in the first and second 3-inch sections in the present experiment (I) with those found in an earlier one (II) in which the exposure had been much longer

SOIL	HYGROSCOPIC COEFFICIENT	EXPERIMENT NO.	INITIAL RATIO	AMOUNT OF WATER ADDED	TIME OF EXPOSURE	RATIO AT END OF EXPERIMENT		FALL IN RATIO AFTER FIFTH DAY	
						1 to 3 in. section	4 to 6 in. section	1 to 3 in. section	4 to 6 in. section
				inches	*days*				
A	13.3	I	1.0	1.00	5	1.9	1.7	0.0	0.0
		II	1.0	2.11	110	2.0	1.8		
B	12.9	I	1.0	1.00	5	1.8	1.6	0.0	0.0
		II	1.0	2.12	69	1.9	1.8		
C	10.5	I	1.1	1.00	5	2.2	1.9	0.1	0.0
		II	1.1	1.21	100	2.1	1.9		
D	10.2	I	1.0	1.00	5	2.4	1.9	0.2	0.0
		II	1.3	0.90	110	2.2	2.0		
E	10.1	I	1.0	1.00	5	2.1	2.0	0.2	0.2
		II	1.0	1.42	100	1.9	1.8		
G	8.2	I	1.0	1.00	5	2.2	2.0	0.2	0.0
		II	1.0	1.58	106	2.0	2.0		
H	7.6	I	1.0	1.00	5	2.4	2.2	0.1	0.1
		II	1.2	1.28	102	2.3	2.1		
I	7.1	I	1.1	1.00	5	2.3	1.9	0.2	0.0
		II	1.2	0.60	102	2.1	1.9		
J	5.6	I	1.0	1.00	5	2.6	2.2	0.5	0.3
		II	1.0	0.89	70	2.1	1.9		
K	3.4	I	1.1	1.00	5	3.7	3.0	1.5	1.1
		II	1.3	0.33	100	2.2	1.9		
L	3.4	I	1.0	1.00	5	2.9	2.7	1.2	1.1
		II	1.3	0.27	68	1.7	1.6		
M	3.3	I	1.1	1.00	5	4.1	3.0	1.7	1.0
		II	1.3	0.35	100	1.4	2.0		

figure 1 a plot of results given by him is shown for soils of varying texture from sands to clay loams. The finer soils represented by the higher curves were allowed to stand 60 days, whereas dripping continued with the sands for nearly 2½ years.

Table 2 is also quoted from the work of Alway (2, p. 35). At a superficial glance, the data here presented would seem to indicate that an equilibrium condition with uniform moisture distribution may have been attained in

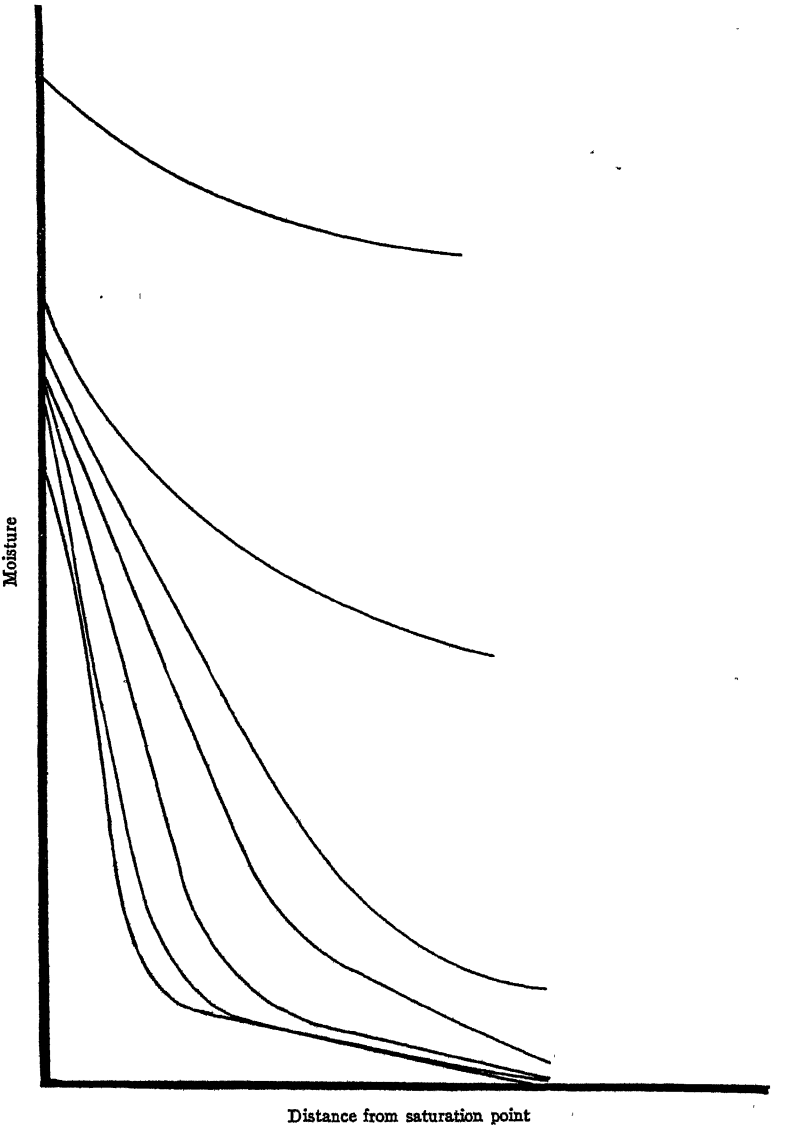

FIG. 1. CURVES REPRESENTING RELATION OF MOISTURE CONTENT TO DISTANCE FROM SATURATION POINT IN DIFFERENT SOILS

the case of soil tubes IV, V, VI, and VII, which had remained standing for 96, 104, 109 and 126 days, respectively, showing but slight variation with the time at every section to the total depth of 30 inches after the 96-day period. The process of readjustment to equilibrium being a very slow process, however, it may be that the actual change during this interval was of the order of

TABLE 2

Ratio of water content to hygroscopic coefficient in soil J, entirely protected from evaporation, but in capillary connection with the earth's soil mass; to the cylinders, each filled with approximately 50 pounds of air-dried soil, there was added 15 pounds of water, after which they were left 31 to 126 days

DEPTH OF SECTION	CYLINDER NUMBER								
	I (31 days)	II (44 days)	III (54 days)	IV (96 days)	V (104 days)	VI (109 days)	VII (126 days)	IV–VII (average 109 days)	IX* Sand at bottom
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	3.1	3.3	3.1	2.5	3.0	2.6	2.6	2.6	3.4
2	3.1	3.2	3.0	2.4	2.8	2.6	2.5	2.6	3.2
3	3.1	3.2	3.1	2.3	2.7	2.5	2.5	2.5	3.2
4	3.2	3.2	3.1	2.4	2.6	2.5	2.5	2.5	3.2
5	3.3	3.2	3.1	2.4	2.6	2.5	2.5	2.5	3.1
6	3.2		3.1	2.4	2.6	2.6	2.4	2.5	3.2
7	3.2	3.2	3.1	2.4	2.6	2.6	2.4	2.5	3.3
8	3.3	3.3	3.2	2.4	2.5	2.5	2.4	2.5	3.3
9	3.4	3.4	3.3	2.4	2.6	2.4	2.4	2.5	3.4
10	3.3	3.4	3.3	2.5	2.6	2.4	2.4	2.5	3.4
11	3.2	3.5	3.4	2.4	2.6	2.5	2.3	2.4	3.4
12	3.5	3.4	3.3	2.4	2.6	2.5	2.5	2.5	3.4
13	3.6	3.4		2.4	2.6	2.5	2.4	2.5	3.5
14	3.6	3.5	3.4	2.4	2.6	2.4	2.4	2.5	3.5
15	3.7	3.6	3.4	2.4	2.5	2.4	2.5	2.5	3.6
16	3.6	3.4	3.5	2.3	2.5	2.4	2.6	2.4	3.6
17	3.7	3.6	3.5	2.3	2.5	2.4	2.5	2.4	3.7
18	3.7	3.9	3.5	2.3	2.6	2.4	2.5	2.4	3.7
19	3.7	3.7	3.5	2.3	2.6	2.4	2.5	2.4	3.8
20	3.7	3.7	3.6	2.4	2.6	2.4	2.5	2.5	3.8
21	3.8	3.8	3.7	2.3	2.5	2.3	2.5	2.4	3.9
22	4.0	4.1	3.8	2.3	2.6	2.4	2.5	2.4	3.9
23	4.2	3.9	3.8	2.3	2.5	2.4	2.4	2.4	4.0
24	4.2	3.9	3.8	2.3	2.5	2.5	2.5	2.4	4.1
25	4.2	3.9	4.1	2.3	2.5	2.5	2.5	2.4	4.2
26	4.4	4.3	4.2	2.3	2.5	2.5	2.5	2.4	4.3
27	4.5	4.5	4.2	2.3	2.5	2.5	2.5	2.4	4.3
28	4.5	4.3	4.2	2.5	2.4	2.5	2.4	2.5	4.6
29	4.6	4.3	4.5	2.5	2.5	2.5	2.4	2.5	
30	4.8	4.3	4.6	2.5	2.5	2.5	2.5	2.5	
Average....	3.7	3.7	3.6	2.4	2.6	2.5	2.5	2.5	3.6

* Column 9 is the mean for four cylinders similar in every way except that connection is made to soil mass through sand and gravel.

magnitude of the probable error of the determinations. It will be noticed that in soil columns I, II and III, which were left to stand 31, 44 and 54 days, respectively, all of which show a decided increase in moisture content with increasing depth, there is very little change in a period of 23 days. The

seven tubes were treated the same way except that I, II and III were taken from an earlier experiment, and since the condition of the natural subsoil into which these tubes drained is not specified, it seems probable that the subsoil may have changed in moisture content, which would readily account for the difference in moisture content in the two series. A moist soil or a gravel or sand at the bottom of the tubes might operate as an effective seal against the downward flow of water by capillarity. The summarized data from four soil tubes which were treated in every way similar to the seven mentioned above, except that they were connected to the subsoil through sand and gravel at the bottom (2, p. 36) have been added as a final column to table 2, and it will be noted that the distribution at the end of a period of 126 days and the average moisture content are almost exactly the same as in tubes I, II, and III, with a decided increase in moisture content with the depth. Had tubes I, II, and III been allowed to stand 126 days, there is no indication that the moisture distribution and the moisture content would have been the same as in tube VII (which stood 126 days) unless we grant that the time interval from the 54th to the 96th day has some singular significance, which, however, is improbable.

Widtsoe (10) defines field moisture capacity as "the percentage of moisture held in field soils to a depth of 8 to 10 feet, when the top foot is saturated," the implied assumption being that equilibrium distribution has been reached. In such distribution, however, the moisture gradient is reversed, showing a decrease in moisture with increasing depth, and, owing to the great depth of the Greenville soil, a state approximating a *steady*, though not an *equilibrium*, state may perhaps have been attained, whereby the dry deep soil carried the moisture away nearly as fast as it would penetrate from a moist surface, although an observation over longer periods of time would perhaps have shown a slow accumulation at all points below the saturation plane.

It is well known that under specified boundary conditions in such phenomena as the flow of heat, a *steady* state is frequently attained with a finite temperature gradient, but such gradient is dependent upon the nature of the boundary conditions. It is possible that a deep dry soil below the tenth foot would maintain a fairly constant moisture content at this depth for a limited time, but as the moisture front advanced into the soil the distance from the saturated surface to the dry boundary would necessarily increase, accompanied by a corresponding change in the distribution. Widstoe claims that the condition specified is not far from the optimum for the Greenville soil and it would seem that a rational basis is thus afforded for a definition of field moisture capacity. However, in addition to specifying the moisture condition at the surface, the depth of the dry-moist boundary should be specified. The proper depth to specify may, however, vary with the type of soil.

The experimental determination of a soil constant as thus defined would be difficult and if adopted it is apparent that a correlation with some other

more readily determined soil constant is desirable. Briggs (6) gives the following equations:

$$c = 2.9w + 21$$
$$c = 1.57e + 21$$
$$c = 4.26h + 21$$

where c is written for the moisture capacity as defined by Hilgard, w for wilting coefficient, e for moisture equivalent, and h for hygroscopic coefficient. It is to be expected that similar relations would hold for a moisture capacity as above or equivalently defined. It is evident, however, that the ratio of c in the above equations to any of the other constants, w, e, or h, is not independent of the character of the soil and it is therefore not surprising that Israelsen (8, p. 23) should get values for the ratio e/c' ranging from 1 to 1.72, and that Alway (1, p. 415) should get values ranging from 1.7 to 4.1 for the ratio c'/h, c' in this case being the moisture content after irrigation. Briggs' equations are admittedly empirical, but the constants he refers to have been defined without ambiguity and the equations may no doubt be applied with fair accuracy to any type of soil. As stated, similar functions may be determined with the field-moisture capacity, however it may be defined; but it cannot be too strongly emphasized, in the light of the foregoing, that the dynamic character of typical soil-moisture phenomena cannot be overlooked in the attempt to define such a soil constant, and in order to make positive progress from a scientific as well as practical point of view, it would seem that fundamental concepts of this character should be carefully considered.

A laboratory study of the dynamic phases of soil moisture is under way at the Utah Agricultural Experiment Station and it is hoped that some information may be made available which will help to rationalize studies in this field.

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THE CARBONATION OF BURNT LIME IN SOILS[1]

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INTRODUCTION

The practice of applying burnt lime to soils has long been followed as one of the fundamental agricultural arts. Particularly is this true in European countries. The scientific principles involved in the practice are, however, of but recent and partial development. During ancient times, the beneficial effects upon soil structure and increased plant growth, following the use of lime, were attributed to the production of a soil condition known as "sweet." From modern investigations, more particularly in the laboratory, it is now known that the benefits derived from the use of lime are due to its effects upon conditions, physical, chemical and biological. The amelioration of unfavorable physical conditions; the eradication of various chemical soil constituents of acid or toxic nature; the supplying, directly or indirectly, of plant-food requirements; and biological betterment have been urged as compelling independent or collective reasons for the application of lime to soils. While the subject of liming, even yet, has had but relatively limited scientific development, the importance of the practical and economical phase of the question has increased to a marked extent. Even in some newly cleared virgin soils, liming is found to be shortly or immediately a profitable treatment; while in the rejuvenation of worn-out and abandoned soils lime is often found to be a prerequisite.

Nevertheless, in some localities even today the need of lime is unrecognized or ignored. In many soils which have long been subjected to cultivation, the continued demand of plants for calcium as a nutrient, together with its loss by leaching, has resulted in a deficiency of lime, which, in some cases, has assumed the proportion of a limiting factor. This is often true in the case of residual limestone soils, formed *in situ*.

With the advent of improved appliances and machinery for tillage, and with the development of more intensive cultivation, the transported calcareous soils of more recent origin are now more rapidily depleted of their lime contents. Better aeration results in greater oxidation of soil organic matter, and it follows that the soil solution will become richer in carbon dioxide. Thus

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a stronger solvent will be furnished for the dissolving and leaching of mineral calcium carbonate, or for the hydrolyzation and leaching of other forms of lime. Data upon this phase of the subject have been reported by Hall (27) who states: "The Rothamsted soil which at the beginning of the Nineteenth Century must have contained something like a hundred tons of chalk per acre has now less than fifty and many other soils which started with a smaller initial stock are beginning to run dangerously short." Additional data concerning the conservation of lime have been reported also from the Hawaiian Sugar Planters' (12), Florida (4), Maryland (6), Cornell (45, 46), and Pennsylvania (47) stations, and by Gilchrist (25) and others.

The development of modern crushing machinery and the advent of by-product limestone has given a healthy stimulus to the use of lime in the form of ground rock. As a result, the term "liming" has become a synonym for application of either burnt lime or ground limestone. In ancient times the soft chalks of Europe were dug and made available by being brought to a state of fineness through exposure to the disruptive action of weathering. In a more restricted way, wood ashes also were utilized, particularly in vineyard cultivation. However, in localities where neither chalk nor marl was available "burning" of limestone constituted the only practice which afforded an extensive source of agricultural lime. Hence, while, prior to more recent times, the question of liming often involved only consideration, pro and con, of the use of the one available form, the burnt oxide, agriculture of the present day has to consider, both economically and scientifically, the question of burnt lime versus ground limestone. As a natural sequence, there have developed diversity of viewpoint and division of opinion as to the relative merits and diverse or parallel activities of these respective forms of lime. A general comparative study of the lime problem embraces a wide range of investigation, for an unbiased judgment requires abundant data upon which to base a thorough understanding of the complex question. A study of the available literature and experimental findings reveals an amazing lack of fundamental and thoroughly scientific data on the problem of differential liming.

The application of either burnt, air-slaked or water-slaked lime to soils is followed by the phenomena of absorption and carbonation in sequence, or in the reverse order. The existing soil moisture conditions and the manner of applying or incorporating will be the deciding factors in determining the order of the changes, as will be shown in this contribution. If the application is in excess of the immediate absorption coefficient, formation of calcium carbonate from the unabsorbed lime will ensue. The term "immediate lime absorption coefficient" is here used because of the fact that it has been shown by the writer (47, 48) that there occurs a progressive decomposition of calcium carbonate and absorption of calcium by soil, when soil and calcium carbonate continue in contact under moist conditions This slow, continued absorption, subsequent to the initial rapid absorption, may continue over a period of years. The phenomenon has also been demonstrated, by Bou-

youcos and Laudeman (5), in a different manner over shorter periods. Therefore, an ultimate quantitative chemical comparison between the activities of burnt lime and natural lime rock in soils may well be based upon periodically determined occurrences of $CaCO_3$. Such a requirement necessitates a careful study of the factors influencing or controlling the carbonation of burnt lime in soils. This dissertation is intended to embody results obtained in carrying out field-exposure and laboratory experiments on this particular phase of the complex lime problem. It is not intended to incorporate those data which have been secured on the somewhat related questions of supposed base substitution, quantitative biological and physical effects, although to some extent these factors are necessarily considered.

HISTORICAL

In the practice of applying lime, the caustic may be so exposed or handled as to become, in large measure, carbonated prior to its contact with the soil. Or, again, it may be converted to the carbonate while lying on the surface of a dry soil or dust mulch, before its incorporation with the body of the soil. Such a condition of dry surface contact would minimize, if not prevent, absorption until admixture of soil and carbonate were effected. It thus becomes necessary first to consider the conversion of oxide and hydrate to carbonate, under conditions where the factor of absorption by the soil is eliminated.

Though qualitatively a well-known chemical reaction, the conversion of CaO or $Ca(OH)_2$ to $CaCO_3$, following exposure, has received but little attention quantitatively. Expressions of opinion are to be found, and some data are to be cited from authoritative sources, however, but few controlled experiments are available for reference. Gillmore (26) states that complete carbonation of some limes may take place within 20 to 25 days in layers approximately one foot in depth, while under the same conditions other limes may require as long as a year to effect complete carbonation. Vicat (68) claims that at the end of one year's time the carbonation of a layer of "fat" lime in free contact with the atmosphere extends to a depth of 0.10 to 0.12 inch. Eckel (13) states that the hydrated lime within the body of wall plaster never undergoes carbonation, because of the protecting action of the $CaCO_3$ layer. Lloyd (44) states that ordinarily when high-grade burnt lime is exposed in the field and slaked under natural climatic influences, large heaps of lime will become pulverized by spontaneous slaking within 4 to 6 weeks. He cites an analysis of a 99 per cent lime so treated as showing 15 per cent $CaCO_3$ and 83.4 per cent $Ca(OH)_2$. Kellner (39) placed compact circular piles of slaked lime under shelter and found after free exposure for 8 weeks, that only 3 per cent of the lime had become carbonated, no further increase being found subsequent to that period. Frear (21) states that his examination of a sample of burnt lime taken 4 inches from the surface of a large pile 10 years old

showed but 10 per cent carbonation. Burchard and Emley (7) report that analyses of 11 samples of commercial fine hydrated lime in bags gave an average of 8.57 per cent $CaCO_3$. The time intervening between hydration and analysis was not given. These writers also gave figures obtained by them in comparative studies of the rate of carbonation of burnt and hydrated lime. Samples of 60-mesh materials were exposed for 10 days and analyzed 6 times during the interval. The carbonation of the hydrated lime was consistently greater throughout, and at the end of 10 days its percentage of CO_2 was 11.25 as compared to 8.73 for the quicklime. These data are particularly interesting when compared with those of tables 3 and 4, in which the reverse is recorded. Feilitzen (15) made the interesting observation that the steam generated in the water-slaking of lime produces a hydrated lime more finely divided than that obtained from air-slaking. The latter gave 52 to 75 per cent of screenings finer than 2 mm. This might be attributed to the granulation following the greater partial carbonation of the air-slaked lime. This observation is interesting in view of the statement by Eckel (13) that if the full required amount of water be added at once to a pure lime the bulk will be increased $3\frac{1}{2}$ times, while air-slaking effects an increase in volume of but 1.7 times that of the burnt lime. Frear (22) cites data obtained through personal correspondence to the effect that 7 per cent of a 40-pound sack of hydrated lime had carbonated at the end of one year, while 14 per cent had been converted into the carbonate form at the end of 2 years. Wheeler (69), reporting upon the composition of air-slaked lime used by the Rhode Island Station, states that the amounts of CaO have varied from 68 to 75 per cent. The writer has made periodic observations of piles of a bushel or less of slaked lime, where it was evident that no appreciable carbonation of the hydrate occurred beyond a thin surface layer, unless sufficient moisture were present to cause extensive cracking and checking of the mass during drying.

Subsequent to the drafting of the manuscript of this thesis, and after completion of the analytical work reported, a most interesting article, by Woodhead (73), has appeared in print. Woodhead studied the reversion of freshly burnt powdered and lump CaO, under different conditions of exposure. Two hundred pounds of freshly burnt lump lime were powdered and subdivided into 100-pound lots. One 100-pound portion was spread on a dry floor and turned and respread daily for 64 days. Residual CaO determinations were made periodically by the 10 per cent sugar solution method of Hendrick (32). The initial CaO content decreased to 63.3 per cent after 10 days; to 57.12 per cent after 20 days; to 52.36 per cent after 30 days; to 47.18 per cent after 40 days; to 45.31 per cent after 50 days and to 39.48 per cent after 64 days. This represented a total CaO decrease of 29.62 per cent, equivalent to 43 per cent of the original CaO. The second 100-pound lot of powdered CaO was immediately dumped outdoors, where it was subjected to weather for a period of 4 months, after which time it was found

to be covered with a crust devoid of CaO. The interior of the pile showed a residual CaO content of 66.92 per cent. The average CaO content determined by the analysis of the mixed crust and interior was found to be 60.83 per cent. This represents a CaO decrease of less than 12 per cent.

Another sample of 200 pounds was exposed in the lump condition. One lot of 100 pounds was bagged and exposed under shelter. After 4 months this sample was mixed and found to contain 50.26 per cent residual CaO as compared to an original content of 81.2 per cent. The second portion of 100 pounds of lump CaO was exposed to the elements for a period of 4 months, at the end of which time the crust gave a residual CaO occurrence of 1.82 per cent, while the interior contained 54.88 per cent CaO. The mixed sample of crust and interior gave a percentage of 38.78 CaO as compared to the original content of 81.2 per cent.

Woodhead's findings, as summarized by him, are:

(1) Powdered lime—dry shed exposure and frequent turnings—deteriorated 43 per cent in 64 days.

(2) Powdered lime—heaped outside, complete exposure—deteriorated 12 per cent in 4 months.

(3) Bagged lump lime—sheltered outdoors—deteriorated 38 per cent in 4 months.

(4) Heaped lump lime—exposed to elements for 4 months—deteriorated 52 per cent.

It would appear that the crust formed over the surface of the powdered CaO exposure served as a watershed and as a layer relatively impervious to an infusion of CO_2. The same protective action of $CaCO_3$ will be shown later to hold for smaller quantities in laboratory studies.

In the discussion following the presentation of Woodhead's paper, Dr. Voelcher, of the Woburn Station, offered data on the subject. These data were secured by comparisons made in 1910 and 1916 upon surplus quantities of lime used in lime experiments at the Woburn Station. The residues were kept in covered wooden tubs.

PER CENT OF TOTAL LIME IN	BUXTON LIME	CHALK LIME	MAGNESIAN LIME	LIOS LIME	OOLITE LIME
1910	92	95	60	70	75
1916	74	80	54	55	65

From these analyses, Dr. Voelcher concluded that less deterioration in burnt lime would be encountered than would be indicated by Woodhead's results.

Considering the carbonation of burnt lime, with reference more particularly to soils, we find some decided opinions as to the phenomenon, but a dearth of data. Heinrich (31) contends that lime reverts to the carbonate very quickly after its application to soils. Storer (63) states, "It is not to be supposed that in the field, reactions due to causticity of the lime can go

on for any long period." According to Frear (19), "There is considerable doubt upon this question. . . . . It is generally conceded that the lime must be carbonated almost immediately after it is spread and harrowed in," and "it is almost necessary to assume that lime cannot long remain caustic after it has been spread and worked into the soil in a finely divided state." Storer (63), citing without reference the work of Heiden, states, "But recent investigations by Heiden go to show that a part of the lime may remain in the soil for years in the soluble form, at least on lands that have been heavily limed. Heiden argues that the lime soluble in water was chiefly in the form of hydrate of lime, because the soil had an alkaline reaction and because he found comparatively little carbonic acid in the solution." Apparently the fact was overlooked that CO_2-treated precipitated $CaCO_3$ and the mineral carbonates of calcium will hydrolyze and give alkalinity indications in both cold and hot distilled water and in absence of free CO_2, even when phenophthalein is used as an indicator. This has been demonstrated by the writer (49).

Fippin (16) writes, "While lime in large lumps air-slacks relatively slowly, when in the fine powder in which it should be incorporated in the soil, the change is rapid. We know of no determinations of the rate of this change in the soil and we do not see how it can be accurately determined in normal soil; but there is much reason to believe that the reversion would be complete in a few weeks at most." The writer has been able to find only one attempt to determine quantitatively by chemical means the rapidity of carbonation of burnt lime in soils. This work was reported by Dr. Wm. Frear in 1899 (20). Nine soils, all being acid in reactions to litmus, were mixed with freshly burnt lime at the rate of 10 gm. per 2000 gm. of air-dried soil. They were then moistened with water and permitted to stand for 10 months, further additions of water being made during the first 3 months. At the end of the 10-month period, the soils were again air-dried and analyzed for CO_2. The analyses when averaged showed that, of the CaO added, 28 per cent had reverted to $CaCO_3$. While it was recognized that the CO_2 analyses did not establish the absence of residual CaO from that applied, it was believed by Frear that, "Considering the relatively large quantity applied, it is difficult to conceive that a very large fraction has not remained in caustic condition." Dr. Frear further states, "The evidence favors the belief that the conversion of caustic lime to carbonates in the soil may be much less rapid than has been heretofore assumed." Quite unfortunately, in this experiment no precipitated $CaCO_3$ checks were included, as parallel treatments. Hence, it is difficult to say how much of the uncarbonated CaO was used to satisfy the tendency of the soil to absorb lime. On the other hand, restricted diffusion of atmosphere to the soil, and probable decreased biological activities as a result of partial sterilization from the excess of CaO, may have depressed the generation of CO_2, thus permitting some of the applied lime to remain as hydrated lime. More recently biological activities have been used at the Rothamsted Station (37) as a means

of indicating the period required for complete reversion of lime to carbonate. It is stated in this work that chemical studies will be reported later (38).

Hoagland and Christie (33), in a recent article, state " . . . . but the assumption is generally made that carbonation quickly takes place, so that the ultimate effect is the same as though the carbonate had been used in the first place." The same authors, in hydroxyl-ion concentration studies, noted a rapid decrease from the immediate and maximum concentration within a few days after additions of CaO to several soils, though the OH-ion concentration continued for a long time in excess of that of the original alkaline soils, and also that of the alkaline soils, which received $CaCO_3$ equivalent to the applications of CaO, which applications were at the rate of 0.07 per cent and 0.28 per cent CaO. The authors state that, "After the first few days the alkalinity decreases in all soils, either because of continued chemical action or of slow reaction with CO_2."

In another experiment the same authors treated an acid fine sandy loam with 0.5 per cent CaO, using an equivalent quantity of $CaCO_3$ as a check. After one year's exposure, "The soil treated with $CaCO_3$ gave an exponent of 8.05, while that treated with CaO gave 11.34. The latter represents an intense alkalinity, and it is clear that complete carbonation did not take place even after a long period of time." The admixtures of lime were throughout the entire body of the soil, and it is assumed that the sampling was representative of the whole mass. From the data offered in tables 32 to 35, it is evident that carbonation takes place much more rapidly in the surface than in the lower stratum of a soil treated with CaO. It is quite possible that a like condition existed in the soils studied by the authors above named. However, the data which will be offered in the cylinder and lysimeter studies strongly indicate that with applications in excess of the lime absorption coefficient more CaO is absorbed by the soil from CaO and $Ca(OH)_2$ treatments than from equivalent amounts of $CaCO_3$. This means that heavy CaO and $Ca(OH)_2$ treatments produce a greater mass of freshly formed silicates. These silicates readily undergo hydrolysis, and to the writer it would seem as reasonable to assume such to be the cause of an excess of OH-ion as to attribute the alkalinity to residual $Ca(OH)_2$ from applied CaO. As a matter of fact, Plummer (54) found, when examining certain soils which were sent to him by the writer, that the OH-ion concentration was very marked as the result of hydrolysis of silicates which had been formed from applied carbonate, though the latter had entirely disappeared many months prior to the ion-concentration studies.

CHEMISTRY OF THE CARBONATION OF BURNT LIME

Historical

There appears to be some confusion in the general usage of the term "air-slaked lime." This fact was taken into consideration by Emley (7). He states that the Bureau of Standards has adopted the term "water-slaked

lime" to designate the product resulting from the hydration of CaO, while the term "air-slaked lime" is used to designate the carbonate end product. The reversion of burnt lime to the carbonate is considered as proceeding through the stages

$$CaO + HOH \rightleftharpoons Ca(OH)_2 \text{ and } Ca(OH)_2 + CO_2 \rightleftharpoons CaCO_3 + H_2O,$$

when exposure is permitted under normal conditions. While it has generally been conceded that the hydration of lime precedes its carbonation, and that this order is due to the fact that the atmosphere affords more of moisture than of CO_2, very few data on the question of relative affinities of H_2O and CO_2 for CaO were available. This phase of the problem has considerable bearing upon the control of experimental conditions in some of the soil studies. In the planning of the experimental work, certain contingencies incident to the sampling, preparing and bottling of the treated soils at successive periods were anticipated. This necessitated the securing of data relative to errors which might be expected to result from carbonation of the hydrate in samples during the process of handling and preparation for analysis. In some instances, of necessity, many hours elapsed between the bottling of the first and last samplings of the large number which were taken at the same period. It was thus essential to submit all of the samples collected at a particular period to treatment which would effectuate conditions that would quickly terminate carbonation. The most feasible procedure seemed to be that of drying the soils in an electric oven, at a temperature which would quickly free the soil containing lime, still uncarbonated, from its dissolved and free CO_2 and at the same time stop the generation of CO_2. It was, therefore, necessary to determine whether, at relatively low temperatures, lime devoid of moisture would cease to be susceptible of conversion to the carbonate. The literature and a few references were supplemented empirically. Mendeleeff (52) states that anhydrous lime will not combine with dry carbonic anhydride, citing Scheele as authority. Schuliachenko is also quoted as stating that the combination does not take place, even at 360°. Mendeleeff further states that, "if the lime is slaked or dissolved, the absorption of carbonic anhydride proceeds rapidly and completely." If by this is meant that dry slaked lime will combine with dry CO_2, that the solid phase of $Ca(OH)_2$ will combine with dry CO_2, the foregoing statement is not susceptible of proof. Veley (66), in 1893, studied "the inertness of quicklime" and pointed out the unreliability of the few observations which had been recorded at that time. Veley demonstrated the inactivity of CaO toward CO_2 and toward SO_2 and the oxides of nitrogen. In Veley's experiments the gases were passed over the lime during the period of ignition. However, working with open platinum crucibles, and using coal-gas for ignition, the writer found an extensive reaction between SO_2 and lime heated to a white heat. This was followed by oxidation to sulphate. Three charges of 0.6 gm. of sulfur-free CaO were ignited continuously for 2 weeks over Bunsen burners. At the end

of that time, the charges had materially increased in weight. Upon test, it was found that the CaO was heavily impregnated with $CaSO_4$. The ignition was repeated on another sample, with the following results. A charge of 0.6 gm. of marble-derived $Ca(OH)_2$, sulfur-free, was ignited continuously for a day and two nights. It was then dissolved and sulfates determined. A weight of 0.0881 gm. of $BaSO_4$, equivalent to 0.0513 gm. $CaSO_4$ was obtained.

In a later article Veley (67) also considers the quiescence of dry chlorine toward CaO. Veley offers in the first-cited article the only comparison originally available to the writer, in which the oxide and hydrate are considered in parallel. Though he does not establish the absence of reaction between dry $Ca(OH)_2$ and dry CO_2, he does show that the amount of water added to CaO is a factor in determining the extent of the reaction within a given time He also shows that the addition of uncombined water to $Ca(OH)_2$ is followed by a marked increase in the tendency of the hydrate to absorb CO_2. He further concludes: "It would, therefore, appear that it is necessary for the reaction that both the hydrate and the carbon dioxide should be dissolved in the water, and the change is incomplete when the gas has only the chance of being dissolved in the water which it succeeds in driving out from the hydrate."

Since most of the experimental work offered in the following pages was completed, the writer has secured a copy of the report of the Columbus (Ohio) Division of Water for the year 1915, in which is incorporated work done by Hoover (34) in a study of the storage of burnt lime. His observations on the changes in volume effected by contact of CaO and $Ca(OH)_2$ with dry and moist air, both with and without CO_2, led to the statement that "the process of hydration takes place first, and . . . . carbonation does not begin to any great extent until after the lime has been completely hydrated." His work further indicated a minimum reaction between $Ca(OH)_2$ and CO_2 in the absence of uncombined H_2O. He also obtained interesting data to the effect that the degree of humidity, as influenced by seasonal changes, is the controlling factor in the speed of the formation of $CaCO_3$, when CaO is exposed in bulk under practical conditions.

Experimental

As to the behavior of dry CaO *toward dry* CO_2. This phase of the problem was considered and tested experimentally in quadruplicate, as follows:

Freshly burnt lime from 0.5-gm. charges of $Ca(OH)_2$, 99.76 per cent pure, were placed in glass-stoppered U-tubes. These were then maintained at a temperature of 105°C. for an overnight period, during which time a current of dried CO_2-free air was drawn through the containers, in order to remove all moisture vapor. The tubes were then cooled and weighed. The weighed tubes were then connected with a CO_2 generator and currents of moisture-free CO_2 were passed over the dry CaO at room temperature for periods of 5 hours.

The CO_2 atmosphere was then displaced by dry CO_2-free air and the tubes again weighed. Drying of the air and CO_2 currents was effected by slow passage through a Greiner-Friedrich absorption tower containing concentrated H_2SO_4. The data in table 1 were obtained.

TABLE 1

Change in weight of tubes containing dry CaO *after passage of dry* CO_2

Determination A	Change of + 0.0028 gm.
Determination B	Change of − 0.0004 gm.
Determination C	Change of − 0.0009 gm.
Determination D	Change of + 0.0012 gm.

Successive daily weighings of the empty tubes and counterpoises showed the above differences to be within limit of error of weighing. A second passage of dry CO_2 for a period of $3\frac{1}{2}$ hours in each case confirmed the above results. During the period of exposure of dry CaO to dry CO_2, 19.4 gm. of the gas passed over each of the charges of oxide. The foregoing data demonstrate that dry CaO and dry CO_2 will not combine chemically at room temperature. This would indicate one of two possibilities, as an essential condition for carbonation—either moisture must be present as a catalytic agency to initiate reaction, or else it is necessary that the reaction proceed through the stage of chemical combination with H_2O, thus: $CaO + HOH \rightleftharpoons Ca(OH)_2$, followed by the combination with CO_2, thus: $Ca(OH)_2 + CO_2 \rightleftharpoons CaCO_3 + H_2O$.

The behavior of dry $Ca(OH)_2$ *toward dry* CO_2. The activity of dry powdered $Ca(OH)_2$ toward dry CO_2 was then studied. Charges of 0.5 gm. of the $Ca(OH)_2$, from which was obtained the CaO used in the preceding work, were dried overnight in an electric oven at 105°C., with aspiration of dry CO_2-free air. Then, after attaining room temperature, the tubes were weighed and a current of CO_2, dried as previously described, was passed over each of the four charges of $Ca(OH)_2$, for a period of 5 hours. Eleven and four-tenths grams of CO_2 were passed through each tube. The data in table 2 were thus secured.

TABLE 2

Change in weight of tubes, containing dry $Ca(OH)_2$ *after 5 hours passage of dry* CO_2

Determination A	Change of + 0.0019 gm.
Determination B	Change of + 0.0022 gm.
Determination C	Change of + 0.0022 gm.
Determination D	Change of + 0.0018 gm.

These data demonstrate either an exceedingly minute chemical reaction between the dry hydrate and the dry gas, or probably no reaction at all. As before stated, blank determinations were run with the counterpoises and these showed that the above amounts are well within experimental error, under conditions encountered from day to day. The small plus indications are due, possibly, to a constant plus error from comparable manipulation.

In this connection it might be contended that the preliminary heating to eliminate moisture would effect a possible molecular change, which might serve to retard carbonation that would have readily ensued with the original hydrate. This was shown not to be the case. In so doing duplicate charges of 0.3 gm. of hydrated lime were placed in 300-cc. Erlenmeyer flasks and exposed to the *outdoor atmosphere* simultaneously with duplicate charges which were first heated overnight in an electric oven at 120°C. Each charge was intimately mixed with dry sawdust, prior to exposure, in order to afford uniform and extensive surface for carbonation. At the end of 6 days, the CO_2 determinations on the four samples gave an average of 16.16 per cent of theoretical carbonation, for both the heated and the unheated charges.

The tendency of reheated $Ca(OH)_2$ *to revert to* $CaCO_3$. However, working with small charges, the effect of long heating at high temperature produced an entirely different result, and one different from what would seem at first thought reasonable to anticipate. Instead of retarding the speed of the carbonate reaction, the additional heating of $Ca(OH)_2$ and its reversion to CaO accelerated the carbonation reaction. This was demonstrated in the following manner: Charges of 0.3 gm. of dry high-grade $Ca(OH)_2$ were ignited for 16 hours over Bunsen burners. At the end of that period, the crucibles were cooled and the charges pestled and thoroughly mixed with dry sawdust. The untreated $Ca(OH)_2$ was weighed, ground and mixed in the same manner, at the same time. Though carefully pestled, the CaO charges could not be considered as strictly comparable in fineness with the pulverulent hydrate obtained by steam slaking. Each of the several charges was then placed in a 300-cc. Erlenmeyer flask and the flasks were exposed side by side for 7 days in a sheltered exterior. At the end of that time, the contents of each flask were subjected to analysis. The ignited charges had extracted almost twice as much CO_2 from the atmosphere as had the unignited ones. This was first done in duplicate, with 0.3 gm., and later checked twice by repetition in triplicate, with both 0.3 gm. and 0.5 gm. charges. In the second and third exposures, constant amounts of acid-washed quartz sand were substituted for the sawdust. The analyses of each series are given in table 3.

The chemically equivalent charges of CaO and $Ca(OH)_2$ functioned differently toward the moisture vapor accessible to the exposures within the flasks. The CaO exerted a chemical attraction resulting in the transition of the CaO to a new chemical compound, $Ca(OH)_2$, while the $Ca(OH)_2$ could only extract moisture through the physical function of surface. It would seem, therefore, that the equal quantities of moisture vapor available to small charges of both CaO and $Ca(OH)_2$ during normal humidity would have effected a greater conversion of hydrate rom solid to solution phase in the compound which had already taken up the amount of water essential for hydration. It appears, however, that the nearly simultaneous hydration and carbonation, possible with the CaO, was responsible for a more rapid formation of $CaCO_3$. Parallel observations with CaO, practically free of silica, as

compared to the same substance after extensive reheating, demonstrated that the additional blasting exerted no depressing effect upon the tendency of the lime to combine with moisture vapor. The experiment, therefore, resolves itself into a comparison between the speed of the formation of $CaCO_3$ from exposures of CaO, and that resulting from simultaneous exposures of chemically equivalent amounts of $Ca(OH)_2$, under the atmospheric conditions encountered. While the differences found might be considered as resulting from a possible molecular readjustment, consequent upon re-heating the hydrated oxide, they are more probably due to the nascency of the reheated oxide and the opportunity for almost simultaneous hydration and carbonation.

TABLE 3

Extent of carbonation resulting from simultaneous outdoor exposures of $Ca(OH)_2$ *and* CaO *obtained by ignition of* $Ca(OH)_2$

	DETERMINATION	PER CENT $CaCO_3$ OF THEORY	
		Unblasted	Blasted
1	A	11.83	31.83
	B	10.72	27.35
	C	10.08	26.71
2	D	17.75	29.91
	E	14.71	30.87
3	F	28.96	36.57
	G	30.12	37.87
	H	30.12	37.49
	Average	19.28	32.33

1. 0.3 gm. exposure with sand, October 12–19.
2. 0.3 gm. exposure with sawdust, October 5–12.
3. 0.5 gm. exposure with sand, October 30 to November 6.

The foregoing results do not bear out the statement of Emley (7), to the effect that $Ca(OH)_2$ will revert to the carbonate more rapidly than will CaO. It seemed quite possible that the question of season, particularly as it affects humidity, might account for the divergence. The further factor of the influence of the size of the charge in its effect upon the moisture and CO_2 vapor pressures in the containers was taken into account. The charges exposed, as shown in table 4, varied from a minimum of 0.3 gm. to a maximum of 2.4 gm. of hydrate. It should probably be emphasized that the hydrate was well slaked and exceedingly pulverulent. Baker's analyzed oxide from burnt marble was used as the source of the hydrate. The oxide was carefully slaked by action of steam generated by the hydration. The mass was then brought to the consistency of a thin paste by further additions of distilled water. It was then dried in a closed flask by heating and aspirating off all

free moisture in a CO_2-free atmosphere. One-half of the number of weighed charges of hydrate were blasted for 30 minutes and the remaining ones were exposed unaltered. The blasted charges were quickly pulverized after cooling. Each charge of hydrated lime and each charge of the blasted oxide was mixed with an asbestos-soapstone mixture and exposed simultaneously. In this way chemical equivalence was maintained and as uniform surface as possible was attained. However, even with careful pulverization the freshly burnt oxide presented far less surface than was offered by the hydrate. In this connection it is of interest to note that the hydrate and oxide charges exposed by Emley were of but 60-mesh fineness.

TABLE 4

Showing extent of carbonation from simultaneous outdoor exposures of $Ca(OH)_2$ *and* CaO *obtained by ignition of* $Ca(OH)_2$

EXPOSURE CONDITIONS			MIXTURE	CHARGE OF ORIGINAL $Ca(OH)_2$	CORRECTED PER CENT CARBONATION	
Period	Season	Atmosphere			Original $Ca(OH)_2$	CaO obtained by ignition of original $Ca(OH)_2$
hours				*gm.*	*Per cent*	*Per cent*
48	June	Humid	Lime and silicate	0.30	31.40	54.37
48	June	Humid	Lime and silicate	0.30	40.54	48.85
48	June	Humid	Lime and silicate	0.30	40.54	51.96
48	June	Humid	Lime and silicate	0.30	39.57	49.54
48	June	Humid	Lime and silicate	0.60	31.85	41.34
48	June	Humid	Lime and silicate	0.60	32.11	40.80
48	June	Humid	Lime and silicate	0.60	32.65	39.41
48	June	Humid	Lime and silicate	1.20	16.59	22.96
48	June	Humid	Lime and silicate	1.20	13.99	21.38
48	June	Humid	Lime and silicate	1.20	12.51	18.73
48	June	Humid	Lime and silicate	2.40	7.82	16.59
48	June	Humid	Lime and silicate	2.40	8.61	20.81
48	June	Humid	Lime and silicate	2.40	9.17	18.01
48	June	Humid	Lime and silicate	2.40	9.97	20.37
Average				1.157	24.53	33.22

The second series of exposures of table 4, made during the month of June and under humid conditions, follow the same trend as those of table 3. However, the influence of humidity is very apparent in its effect upon the actual amount of carbonation effected. With 7 days of exposure during October, an average of the carbonation attained by five 0.3-gm. and three 0.5-gm. charges of unblasted hydrate gave 19.28 per cent as against 32.33 per cent for the blasted hydrate as was shown in table 3. On the other hand, with 48 hours exposure during June, the average per cent carbonations of 41.44 and 54.38, respectively, for the unblasted and blasted hydrates were attained. The influence of moisture vapor in the conversion of the hydrate from the

solid to solution phase is very marked. It is interesting to observe that with the increase in charges the carbonation results become less uniform. This decrease in concordance with increase in charge is particularly noticeable to the analyst.

In each of the 14 simultaneous exposures of table 4 the oxide, obtained by re-heating the $Ca(OH)_2$, gave a more extensive carbonate formation. The larger actual amount of CO_2 absorbed by the 2.4-gm. charges, as compared with the 0.3-gm. charges, indicates that the smaller charges did not effect complete fixation of the amounts of CO_2 which were diffused into the flasks and over the surfaces of the charges.

In a further comparison, the factor of re-heating the oxide was eliminated. In this set Baker's analyzed CaO, derived from marble was compared with $Ca(OH)_2$ derived from the same, burnt lime. The complete hydration of the oxide was accomplished in the same way as described in referring to the exposures of table 4. The results of the third exposure are given in table 5. In this experiment it was intended to expose both chemical equivalences and equal weights of the oxide and hydrate. However, in the exposures of June 30 and July 1, the CaO charges were but 86.33 per cent of the equivalence represented by the $Ca(OH)_2$ charges. This inadvertence was caused by the weighing of hydrate charges from another sample of hydrate. The values of the two substances were ascertained by solution and titration of excess of acid. The amount of CO_2 held by each substance was also determined and correction made therefor. In the charges of June 3 and 5 exact chemical equivalence was secured while the exposures of June 10 were made with equal weights.

In the two exposures of the 0.3-gm. and 0.6-gm. charges of hydrate and the 0.2091-gm. and 0.4182-gm. charges empirically determined to be of but 86.33 per cent equivalence, the same relationship was found, as was shown in tables 3 and 4. The first exposure was made under conditions of excessive humidity while the second was made under normal conditions. The average of 23.44 per cent for the first two CaO exposures as compared to 14.52 per cent for the corresponding exposures of $Ca(OH)_2$ represent a very considerable analytical difference. It should also be remembered that the CaO exposures in these two instances represented but 86.33 per cent of the chemical equivalence of the hydrate. It should also be stressed that the pulverulent hydrate, made by steam, engendered by the action of hydration, was considerably finer than the oxide, though the latter was carefully pestled.

As in the case of the reburnt and original hydrate comparisons, it is particularly interesting to note the extent of carbonation of the eight charges of hydrate and the eight charges of chemically equivalent oxide under the 7-day periods of exposure with normal humidity during October, as contrasted with extent of carbonation shown by similar charges during the more humid weather of June and July. The averages of 19.28 per cent and 32.33 per cent carbonation for $Ca(OH)_2$ and CaO, respectively, from a 7-day period

of exposure during October, are comparable with corresponding averages of 14.52 per cent and 23.44 per cent from eight charges of each $Ca(OH)_2$ and CaO during June and July exposures averaging but 32 hours.

In the case of the larger exposures of table 5 we have, however, the reverse of the findings with the lesser charges. The exposures of 1.9372 gm. of CaO

TABLE 5

Additional simultaneous exposures of CaO *and* $Ca(OH)_2$ *in amounts chemically equivalent and also in equal weights. Lime charges mixed with* CO_2*-free siliceous materials*

EXPOSURE		AMOUNT OF CHARGE		HUMIDITY	CORRECTED FOR PER CENT CARBONATION	
Date	Period	CaO	$Ca(OH)_2$		CaO	$Ca(OH)_2$
	hours	*gm.*	*gm.*		*per cent*	*per cent*
June 30	20	0.2091*	0.3000	Excessive	18.93	13.70
June 30	20	0.2091	0.3000	Excessive	18.19	13.54
June 30	20	0.2091	0.3000	Excessive	28.98	15.78
June 30	20	0.2091	0.3000	Excessive	18.75	14.66
July 1	44	0.4182*	0.6000	Normal	26.13	14.22
July 1	44	0.4182	0.6000	Normal	27.90	15.82
July 1	44	0.4182	0.6000	Normal	22.97	12.93
July 1	44	0.4182	0.6000	Normal	25.66	15.49
July 3	24	1.9372	2.4000	Normal	1.43	2.14
July 3	24	1.9372	2.4000	Normal	1.91	3.00
July 3	24	1.9372	2.4000	Normal	1.39	3.00
July 3	24	1.9372	2.4000	Normal	1.28	2.08
June 5	20	2.0505	3.0000	Normal	2.80	4.18
June 5	20	2.0505	3.0000	Normal	2.80	4.76
June 5	20	2.0505	3.0000	Normal	2.65	5.17
June 5	20	2.0505	3.0000	Normal	2.63	5.62
June 10	48	3.0000	3.0000	Normal	6.15	8.88
June 10	48	3.0000	3.0000	Normal	5.73	7.53
June 10	48	3.0000	3.0000	Normal	7.16	9.38
June 10	48	3.0000	3.0000	Normal	4.42	7.57
Average of 0.3-gm. and 0.6-gm. charges, CaO giving greater carbonation					23.44	14.52
Average of 2.4-gm. and 3.0-gm. charges, $Ca(OH)_2$ giving greater carbonation					3.36	5.28

* 0.2091-gm. and 0.4182-gm. charges of CaO represented but 86.33 per cent chemical equivalence of 0.3-gm. and 0.6-gm. $Ca(OH)_2$ charges, respectively, of $Ca(OH)_2$.

and 2.40 gm. of $Ca(OH)_2$, amounts emperically determined as being chemically equivalent, were made by one analyst, while the chemically equivalent charges of June 5 and the equal-weight charges of June 10 were made by a second analyst. The average per cent carbonation effected by the twelve heavy charges of CaO amounted to 3.36, while the corresponding hydrate charges effected carbonation to the extent of 5.28 per cent. The explanation

for this reversal would seem to be that the oxide charge, in its initial period of exposure at least, would be sufficiently large to take into chemical combination, as water of hydration, most of the diffused moisture vapor, thus leaving but little to effect change of the newly formed hydrate from solid to solution phase. This would not, however, prevent localized occurrences of the dissolved hydrate, as is evidenced by the fact that some, though restricted, carbonation took place.

The catalytic action of film moisture in initiating reaction between $Ca(OH)_2$ *and* CO_2. The failure of the dry hydrate of lime to undergo carbonation when exposed to currents of dry CO_2 suggested the possibility of a need of moisture to effect catalysis. As showing the action of moisture in such slight amounts as represented by the moisture film resistant to aspiration of dry air, the following figures are of interest. Equal amounts of CaO, as hydrated oxide, were placed in each of eight glass-stoppered U-tubes. Four of these tubes were subjected to aspiration overnight with dry CO_2-free air.

TABLE 6

Showing carbonation of $Ca(OH)_2$ *as initiated by the film moisture of the weighing tubes*

	OVERNIGHT ASPIRATION AT 105°C, FOLLOWED BY 3½ HOURS' EXPOSURE TO A CURRENT OF DRY CO_2	OVERNIGHT ASPIRATION AT 25°C, FOLLOWED BY 3½ HOURS' EXPOSURE TO A CURRENT OF DRY CO_2
	Increase	Increase
	gm.	*gm.*
Determination A	0.0019	0.0238
Determination B	0.0022	0.0238
Determination C	0.0022	0.0238
Determination D	0.0018	0.0240

Four other tubes were treated in the same manner except that the tubes were maintained at 105°C. during the overnight aspiration. A current of dry CO_2 was then passed slowly through each of the tubes at room temperature during a period of 3½ hours.

The data of table 6 are corrected for the respective determined amounts of water liberated and aspirated after partial carbonation of the hydroxide. These data show that the film moisture, residual from the overnight aspiration with dry air at room temperature, is sufficeint to produce appreciable dissociation of the hydrate followed by determinable carbonation. The passage of dry CO_2 through the apparatus was very slow and additional water was thus made available to effect further dissociation, as the carbonation proceeded. The carbonate conversion effected in this way represents but 13.44 per cent of theory. Thus the rapidity of the actual conversion of the calcium hydrate from solid to solution phase governs the speed of the carbonate reaction. This may prove to be an important factor in determining the speed of the carbonation of lime lying on the surface soil, in that

the prevailing humidity would affect the amount of moisture available for the dissolving of the hydrate.

The hydration of freshly burned CaO *in a moist atmosphere.* As bearing on certain comparisons between the carbonation of CaO and that of $Ca(OH)_2$ in soils, which will later be considered, it was desirable to ascertain to what extent of theory and with what speed the conversion of oxide to hydrate would transpire in the presence of excessive amounts of moisture vapor. This was done under the following conditions.

The charges of table 1 were subjected for 18 hours to a current of CO_2-free air which was saturated with moisture by circulation through distilled water in a Greiner-Friedrich absorption tower at 25°C. The longer period of contact was given in order to insure an amount of water approaching more nearly the quantity of CO_2 passed over the oxide. The water passed over each oxide charge amounted to 1.9378 gm., of which an average of but 0.1095 gm. was absorbed. After passage of moist air, the tubes were again restored to original dry conditions and weighed with the results set forth in table 7.

TABLE 7

Change of weight of CaO *containing tubes after passage of moist* CO_2*-free air*

Determination A	Increase of 0.1056 gm.
Determination B	Increase of 0.1080 gm.
Determination C	Increase of 0.1094 gm.
Determination D	Increase of 0.1148 gm.

Allowing for CO_2 impurities, the hydration calculates to approximately theoretical requirement. The data above demonstrate that the conversion to the hydrate is very rapid when sufficient atmospheric moisture vapor is available.

Relative activities of currents of dry and moist CO_2 *upon charges of dry* $Ca(OH)_2$. The influence of an atmosphere saturated with reference to CO_2 and H_2O, in affording opportunity for chemical reaction between the strongly dissociated, moist, $Ca(OH)_2$ and the hypothetical acid, H_2CO_3 was next studied. The dry $Ca(OH)_2$ was placed in glass U-tubes and subjected to a current of moist CO_2 for a period of $3\frac{1}{2}$ hours. The current of CO_2 was saturated with moisture by slow circulation through Greiner-Friedrich absorption towers. The moist atmosphere, together with the chemically liberated H_2O was then displaced from the tubes by means of overnight aspiration with dry air. The tubes were then weighed and correction applied for the theoretical moisture liberation.

The increase in CO_2, shown in table 8, represents on the basis of theory a percentage carbonation of 35.58. This does not mean, however, the completion of the total carbonation that could be effected in a given time under the chemical conditions imposed; for, as will later be shown, the apparent attainment of chemical equilibrium is due to the protective coating of carbonate which surrounds the enclosed nuclei of hydrate.

TABLE 8

The action of dry and moist currents of CO_2 *when passed over* $Ca(OH)_2$ *for* $3\frac{1}{2}$ *hours*

	GAIN IN CO_2	
	Dry gas	Moist gas
	gm.	*gm.*
Determination A	0.0019	0.0829
Determination B	0.0022	0.0834
Determination C	0.0022	0.0848
Determination D	0.0018	0.0823

METHODS OF ANALYSIS USED IN SOIL STUDIES

In the work embodied in this thesis, the speed of the carbonate reaction was determined by periodic analyses to establish the extent of accumulations of calcium carbonate resulting from applications of CaO and $Ca(OH)_2$, under the several conditions and with varying amounts. This necessitated the making of a large number of CO_2 determinations. The method followed was that of the liberation of carbonate CO_2 by treatment with HCl, 1 plus 9, and the determination of the liberated CO_2 according to the procedure advanced by MacIntire and Willis (50 a, b).

In the analyses of the 2 and 8-ton treatments of the field cylinders, the absorption of the liberated CO_2 was accomplished gravimetrically through the use of soda-lime and sulfuric acid tubes. In the case of the heavier treatments of the tanks sodium hydrate solution was used to effect absorption of the liberated CO_2. At first the amount of CO_2 absorbed by the hydrate solution was determined by the double titration procedure of Brown and Escombe as perfected by Amos (1). This method of absorption extracts all CO_2 and gives very good results in the case of small amounts of carbonates formed from the CaO of the lighter treatments. However, with heavy applications, it was found that the excessive CO_3 ionization of the carbonate solution resulted in an indefinite endpoint and a tendency toward low results. This source of error was noted also by Lunge (41). Later the same writer (42) advocated the use of NaCl to obviate the difficulty. This tendency necessitated either the use of a factor or the addition of salt to the absorbent solution; or else the temperature precautions described by Lincoln and Walton (43). The method of absorption advocated by Tacke (64) in his lime-requirement method was then substituted. This procedure involves the absorption of the CO_2 gas in NaOH, or KOH, followed by precipitation of the normal carbonate by addition of $BaCl_2$ and subsequent determination of residual hydrate. The absorption towers were washed out with CO_2-free water and the washings plus $BaCl_2$ solution made to a volume of 500 cc. After the settling of the barium carbonate, a 200-cc. aliquot of the clear supernatant solution was drawn off and titrated, phenolphthalein being used as an indicator. One-half normal NaOH absorbent solutions were used in the analyses reported in this thesis.

Persistence of hydrated lime in soils

There appears to be no unanimity of opinion as to the persistence of lime in the hydrated form, subsequent to its incorporation with the soil. Since there exists such a great difference between the solubility of $Ca(OH)_2$, and that of $CaCO_3$ derived therefrom, it is to be expected that the function of a definite amount of lime in a soil will be governed by the form in which it remains in the soil. Granting that a large amount of lime has accumulated in a soil over a period of years, from a series of applications of reasonable amounts of CaO or $Ca(OH)_2$, it is difficult to assume that the free water of the soil would contain the hydroxide because of the continued generation of CO_2, which is to be found in both soil water and soil atmosphere. There is also a great difference in the ionic dissociation of equal amounts of dissolved lime occurring in the relatively dilute soil solutions of the two forms of lime. These inherent differences are of importance from both chemical and biological viewpoints and the question of the continued presence of calcium hydrate in soil, treated repeatedly with ordinary amounts of lime, or with one excessive application, is one of practical, as well as academic significance.

Heiden (30) maintains that $Ca(OH)_2$ remains as such in soil for long periods. Ehrenberg (14) asserts that Heiden's contention is not sustained. It seemed well, therefore, to consider the assumption as unproved, because of Heiden's fallacious reasoning and an effort was made to obtain trustworthy data bearing upon this point. For this purpose there was utilized a field soil which has been limed at frequent intervals during a long period. A soil well adapted to such a study is that of the Pennsylvania Station plats which have received applications of burnt lime semi-annually since 1881. This soil is of limestone origin, and is classified as a silty clay loam of the Hagerstown series. Commencing in 1881, and until very recently, 2 tons of finely ground, unslaked burnt lime were applied each year to each of two plats, numbers 22 and 23, of the tier planted at that time to corn. Recently, however, lime has been slaked prior to its application. Through the courtesy of Prof. C. F. Noll, the writer has had access to the records of the lime plats; while Prof. J. W. White has very kindly furnished freshly taken samples for the analytical work to be described.

Beginning in 1881 and extending through 1915, the following number of pounds of high-grade CaO have been applied to the respective tiers: tier 1, plat 22, 36,500 pounds, plat 23, 36,000 pounds; tier 2, plat 22, 32,500 pounds, plat 23, 36,000 pounds; tier 3, 36,000 pounds for both plats 22 and 23; tier 4, 36,000 pounds, for both plats 22 and 23. The soils of those plats were sampled for analysis in the fall of 1915, 40 borings having been taken from each of the plats of tiers 1, 2 and 3; while 196 borings were secured from each of the two plats of tier 4. The samples used for this study, therefore, represented four series of two plats each. One plat, no. 23, of each pair from the same tier had received lime alone, while the other, no. 22, had received a

supplementary biennial treatment of 6 tons of manure. The four pairs of samples represented respective intervals of less than 1, 2, 3 and 4 years since the application of lime, while the same pairs represented as maximum periods of contact between soil and lime 35, 34, 33 and 32 years, respectively. It should be noted that, even after more than 30 years of cropping, most of the untreated check plats, and many of the fertilized plats, of these tiers were still either slightly alkaline or neutral or else the lime requirement was very low, as indicated by the Veitch method (65). This was shown by Gardner and Brown (24). Hence, it would not be expected that so large a part of the applied lime would be absorbed, in the sense in which the term "absorption" is used as applied to soils, as would be expected if the soil were now distinctively acid or of high lime requirement. However, from ultimate analyses and $CaCO_3$ determinations on these plats in 1911, the writer (47) showed that the increase of $CaCO_3$ from CaO and $Ca(OH)_2$ treatments would not account for the accumulation of calcium. Of the lime remaining in the lime-treated plats, about 35 per cent occurs in forms other than carbonates. Since the same relationship obtained also in the plat treated with ground limestone, it did not at that time seem reasonable to consider the discrepancy between accumulated CaO and the increased $CaCO_3$ content as possibly being due to uncarbonated $Ca(OH)_2$. If only burnt or slaked lime had been applied it might have been assumed that the above-cited data confirmed the contention of Heiden (30) as to the long-continued occurrence of caustic or hydrated lime in soils. Moreover, since the soils contained a large number of pea-sized lumps of lime, it seemed possible that such a contention might be valid, because of possible incomplete carbonation of such lumps. If this were true, pulverization and further exposure would be expected to give an increase in carbonate content. In order to throw light upon this point, the following experiment was carried out. The eight samples from the lime plats were dried in agate ware pans at 105°C. It has already been pointed out that heating to 105°C. had no determinable effect upon the tendency of $Ca(OH)_2$ to revert to the carbonate. All of the dry soil passed through a 2.5-mm. sieve, which, however, stopped a large number of lime lumps. These were added to the soil and the entire sample, amounting in each case to approximately 2 quarts, was then pulverized immediately and passed through a 1-mm. sieve, thoroughly mixed and tightly bottled. Six charges of 25 gm. each were then weighed from each of the eight samples. Each of these charges was then mixed with an equal volume of pure quartz sand and left exposed in shallow tumblers for a period of 3 weeks. In another series, charges of the same weight were placed in 300-cc. Erlenmeyer flasks, which were then filled with moist CO_2 gas and permitted to stand one week, after which time the CO_2 was aspirated off and the samples were again exposed to the atmosphere for a few hours. The two treated series were then analyzed for carbonate CO_2. The analyses, representing from 40 to 60 determinations per series, are given in table 9.

The data of table 9 show that no increase of carbonate resulted after further exposure of the pulverized soil to air, or to an atmosphere of CO_2. This proved conclusively that no $Ca(OH)_2$ was present as residual from either the accumulated earlier applications or the relatively recent ones. The analyses further demonstrate that under the present prevailing conditions in this particular previously heavily limed soil, the carbonation or neutralization of 4000 pounds of CaO per acre was completely effected within a period of 6 months after the application. As supplementing the foregoing tests, one lump of lime weighing about 2.7 gm. was taken from one of the samples of soil and pulverized. A part of the sample was then analyzed in duplicate for CO_2, and another part was treated with carbonated water and

TABLE 9

Complete carbonation of all CaO *added to the Pennsylvania Station plats from 1881 to 1915, inclusive*

TIER	CROP, YEAR OF SAMPLING	PLOT NUMBER	TREATMENT	CALCIUM CARBONATE		
				After grinding, no exposure	After grinding and exposure to atmosphere for 3 weeks	After grinding and exposure to CO_2 for 1 week
				per cent	*per cent*	*per cent*
1	Oats	22	Lime and manure	1.285	1.295	1.295
1	Oats	23	Lime alone	1.173	1.178	1.170
2	Wheat	22	Lime and manure	1.053	1.078	1.074
2	Wheat	23	Line alone	1.515	1.529	1.508
3	Grass	22	Lime and manure	1.335	1.335	1.320
3	Grass	23	Lime alone	1.314	1.313	1.317
4	Corn	22	Lime and manure	1.165	1.164	1.141
4	Corn	23	Lime alone	1.220	1.202	1.203
Average of 40 or more determinations				1.258	1.261	1.254

then analyzed. The percentage of CO_2 in the original portion was found to be identical with that of the CO_2-treated portion. Therefore, in this heavy clay soil containing a large accumulation of lime, no caustic or hydrated lime remained uncarbonated either on the surface or in the soil.

The differences established by the analyses show a lack of uniformity in the composition of the soils of the respective tiers. Furthermore, considering each tier separately, the analytical results in the case of the large plats, adjacent and limed in the same manner, are divergent, in spite of most careful sampling. This fact shows that positive deductions should not be drawn from small differences in the analyses of the soils of these plats in an attempt to demonstrate the effect of burnt lime upon the composition of the soil. Yet smaller analytical differences in the soil of these plats have been

used as the basis of a contention that these plats demonstrate the chemical "burning up" of soil humus and upon such a meagre basis this has been widely taught.

Comparison between the carbonation of $Ca(OH)_2$ *on the surface and when mixed with 1 inch of dry soil*

The first laboratory experiments upon carbonation were for the purpose of studying surface carbonation, as compared to that effected after incorporation of the hydrated lime with dry soil to a depth of 1 inch. The experimental conditions effected were intended to simulate those incident to a surface mulch on the field. It was hoped to determine the speed of carbonation of hydrated lime left on the surface of the dry soil and compared with that taking place when lime is harrowed into the dry soil mulch, which would naturally be relatively inactive as a chemical absorbent. Two alkaline soils were used—a Cumberland brown loam of fair fertility, and a gray residual silt loam of limestone derivation. The Cumberland loam was secured from a spot adjacent to experimental plats of the University of Tennessee Agricultural Experiment Station on the banks of the Tennessee River, near Knoxville. The soil was of alluvial origin, derived partly from a dolomitic limestone. The silty loam was formed *in situ* from Chickamauga limestone and was obtained about six miles from Knoxville, from a tract utilized in coöperative experiments. Nearly two years previously these two soils were used for pot experiments which called for definite applications of $CaCO_3$ in excess of their lime requirement as indicated by the Veitch method (65). The procedure was as follows.

Paraffined paper cartons, 2 inches square and $1\frac{1}{4}$ inches high, were used as containers for charges of soil and lime. Ninety grams of soil, or enough to give 1 inch in depth, were placed in each carton. To each carton, $Ca(OH)_2$ was applied at the rate of 8 tons per surface acre, or 6.1263 gm. per carton. The lime contained 0.82 per cent of CO_2 prior to exposure. In one series the same amount of lime was distributed evenly over the surface of each carton, while in the other admixtures of the entire amounts of lime and soil were made in each carton. All of the cartons were then exposed at the level of the surface soil in a small covered, but well ventilated, outdoor structure. The exposures were made during November and December, 1914. At successive intervals, the soils in the cartons were analyzed. The total weight of the content of each carton was obtained at the time of analysis. The entire content of each carton was then finely ground, and fractions of one-tenth, by weight, were used for analysis. The total gain of CO_2 for each carton was then calculated and correction applied for the CO_2 content of checks. The percentage of carbonation was then determined by comparison with the theoretical amount. The results are embodied in table 10.

The data of table 10 show that the surface applications of hydrated lime reached equilibrium in a short time without complete carbonation having taken place. These layers of lime, about $\frac{3}{16}$ inch in depth, apparently developed protecting coats of $CaCO_3$ around the minute particles of $Ca(OH)_2$, thus delimiting further contact with moisture and CO_2. For, as the data show, a decided increase in carbonation resulted when the applied lime was removed, ground to extreme fineness, and subjected to further exposure.

TABLE 10

Carbonation of $Ca(OH)_2$ *applied to surface of a dry soil and that resulting from its incorporation with 1 inch of the same dry soil*

SOIL	LIME TREATMENT	TIME OF EXPOSURE	CO_2 FOUND	CO_2 INCREASE	CO_2 THEORETICAL TOTAL CARBONATION	CARBONATION	PER ACRE SURFACE
		weeks	*gm.*	*gm.*	*gm.*	*per cent*	*pounds*
Cumberland loam	Spread	1	0.1352	0.1135	0.3635	31.2	4992
	Spread	2	0.2090	0.1872	0.3635	51.5	8240
	Spread	3	0.2130	0.1912	0.3635	52.6	8416
	Spread	4	0.2121	0.1903	0.3635	52.3	8368
	Ground and respread	6	0.2748	0.2497	0.3635	68.7	10992
	Ground and respread	7	0.2768	0.2517	0.3635	69.2	11072
Cumberland loam	Mixed	1	0.0711	0.0493	0.3635	17.4	2784
	Mixed	3	0.1265	0.1047	0.3635	28.8	4608
	Mixed	5	0.2134	0.1916	0.3635	52.7	8432
	Mixed	6	0.2255	0.2004	0.3635	55.1	8816
	Mixed	7	0.2350	0.2099	0.3635	57.7	9232
Silty loam	Spread	1	0.1283	0.1132	0.3635	31.1	4976
	Spread	2	0.2000	0.1848	0.3635	50.8	8128
	Spread	3	0.2026	0.1874	0.3635	51.6	8256
	Spread	4	0.2022	0.1870	0.3635	51.4	8224
	Ground and respread	6	0.2814	0.2629	0.3635	72.1	11536
Silty loam	Mixed	1	0.0557	0.0405	0.3635	11.1	1776
	Mixed	3	0.1309	0.1157	0.3635	31.8	5088
	Mixed	5	0.1984	0.1833	0.3635	50.3	8048
	Mixed	6	0.2112	0.1928	0.3635	52.9	8464
	Mixed	7	0.2295	0.2110	0.3635	57.9	9264

The lime incorporated with the dry soil carbonated more slowly than that applied to the surface. The data indicate that were it deemed desirable to insure complete carbonation of any lumps of $Ca(OH)_2$, prior to its admixture with the soil, it would be advisable to practice dragging, or rolling, or to disturb the surface layer in some such manner. However, the conditions of the experiment were not absolutely comparable with those of the field, in that no moist CO_2-laden soil underlay the treated soil, or rather simulated

soil mulch. Field conditions might result in a greater supply of moisture and CO_2 being made available for carbonation because of diffusion of soil atmosphere. As a matter of fact, such proved to be the case under field conditions. This will later be considered in the light of the data of tables 45,

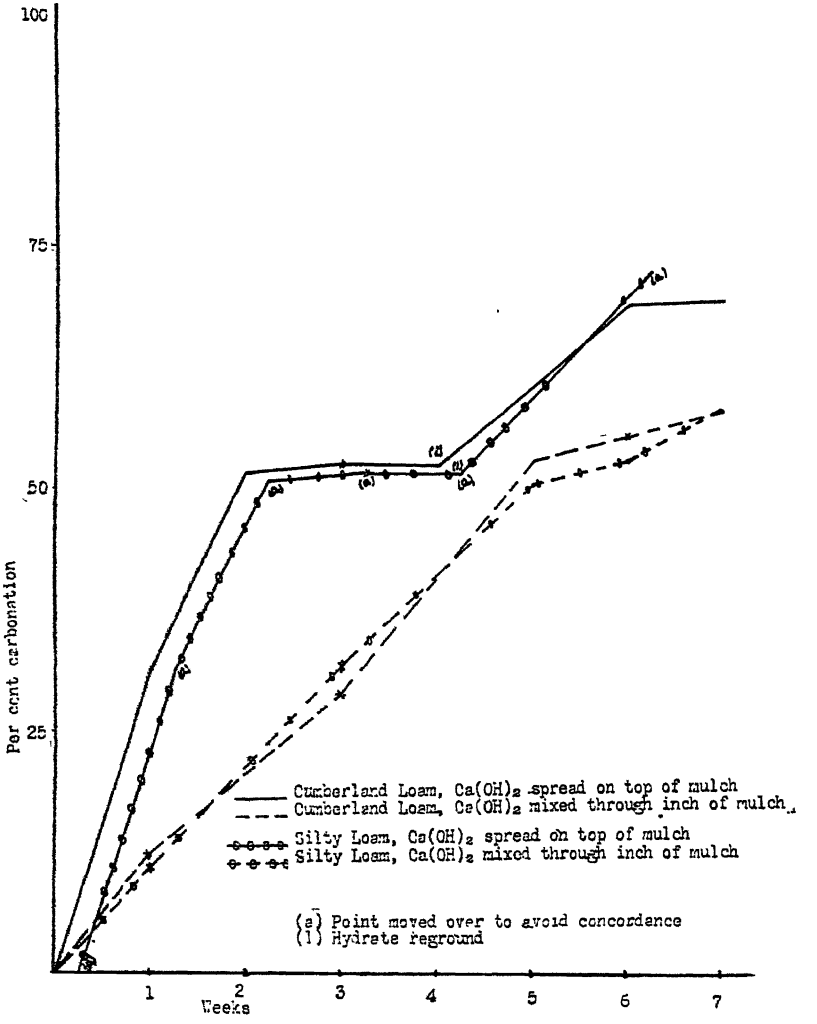

FIG. 1. SURFACE AND INTRA-MULCH EXPOSURES OF $Ca(OH)_2$ (TABLE 10)

46 and 47. While this might affect the speed of the carbonate reaction encountered in both surface and mulch treatments, it would not be expected to change the relationship in the extent of carbonation effected by the two methods of application, surface exposure and incorporation with soil mulch.

Hannen (28) states that diffusion of soil atmosphere is dependent upon size of pores, texture and moisture content. Thus, in the case of sandy and open soils there might be appreciable diffusion of moisture and CO_2 to a layer of lime incorporated in or overlying a dry mulch, while in the case of a fine soil, its absorptive properties, particularly those colloidal, would have a greater retentive influence upon the CO_2. From their studies upon the soil atmosphere, Russell and Appleyard (58) concluded that there exists no close correlation between the changes in soil atmosphere and the aspirating action of winds. In practice, the aspirating effect of wind upon the underlying moist and CO_2-laden soil would be very probably minimized by the presence of a mulch, such as was simulated by the use of the dry soil in the preceding experiment.

FIELD CYLINDER STUDIES

The first field study involved comparisons between carbonation resulting from chemically equivalent amounts of CaO and $Ca(OH)_2$, mixed throughout an acid loam soil. The soil utilized in this experiment was the brown loam used in the preceding mulch exposure experiment and previously described. The preliminary studies of tables 1 to 8 show, however, that the oxide would quickly derive from the soil the moisture essential for hydration. Hence, it is probable that the use of the burnt oxide was simply equivalent to increasing the number of $Ca(OH)_2$ treatments included in the study. On the other hand, in the preliminary studies, wherein the oxide and hydrate were exposed at the same time and place, it was shown that the probable simultaneous taking up of H_2O and CO_2 by the oxide resulted in a more rapid conversion to carbonate, than was effected by the hydrate where only the absorption of CO_2 was essential to the formation of carbonate.

Chemical equivalence of CaO, $Ca(OH)_2$ and $CaCO_3$ treatments was determined by dissolving each of the substances in an excess of acid and titrating back the excess. Chemically equivalent amounts of pure precipitated $CaCO_3$ were used as checks. All materials applied were weighed and sealed in bottles in the laboratory. They were opened immediately before mixing with the soils of the field cylinders. The treatments were based upon equivalence of 4,000 pounds and 16,000 pounds of CaO per acre 2,000,000 pounds of soil, approximating 8 inches in depth. Some of the CaO treatments were unsupplemented, while others received supplementary treatments of finely chopped dry barnyard manure at the rate of 12, 30 and 48 tons per acre 2,000,000 pounds of soil. The burnt lime used was approximately 97 per cent CaO, and had been recently burned. The hydrated lime was obtained by the water-slaking of the same burnt lime. Related studies to determine the difference between the ability of the soil to effect the decomposition of ground limestone, as compared to its ability to decompose the more finely divided precipitated $CaCO_3$, demonstrated that the latter form should be used as a check in determining the conversion of the applied lime and hydrate to the carbonate.

In the entire experiment, of which the carbonation studies were but a part, about 15 tons of ¼-inch-screened and thoroughly mixed soil were handled. The preliminary mixing was done by 9 men working in a circle around the soil heap. When the laboratory sample was air-dried, approximately 97 per cent passed through a 0.5-mm. sieve.

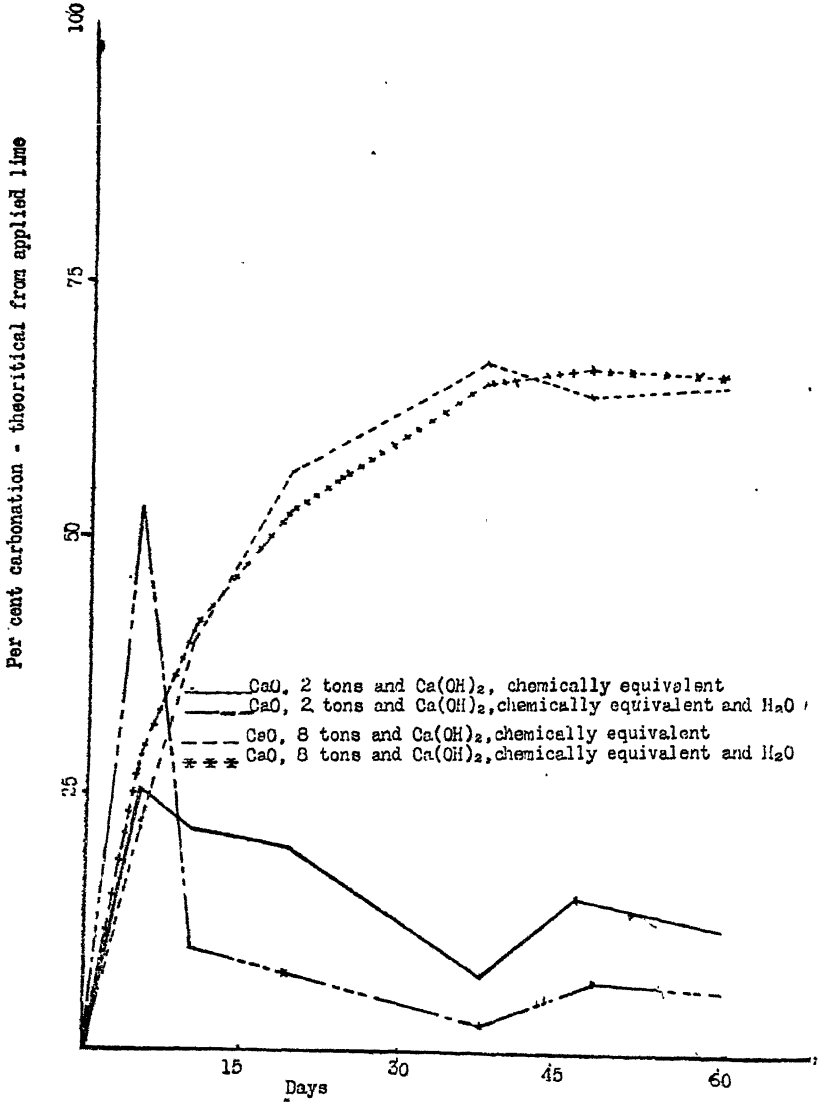

FIG. 2. SPEED OF CARBONATION OF THE CaO AND $Ca(OH)_2$ APPLIED TO ACID CUMBERLAND LOAM IN THE CYLINDERS (TABLES 11 TO 20)

Cloudy, misty weather prevailed during the handling of the soil, and its transfer from a nearby source to cylinders was accomplished without any determinable change in moisture. There was approximately 15 per cent of moisture in the soil at the time of placing and treatment.

The cylinders used as containers for the treated soil were 12 inches in depth and embraced an area of 1/10,000 acre. They were part of a set comprising a total number of 128. Each cylinder received enough moist soil to furnish 200 pounds of moisture-free soil. This was placed as follows: In each cylinder was first placed, untreated, one-fourth of the total amount to be placed, equivalent to 50 pounds of moisture-free soil. This rested upon the

TABLE 11

A field cylinder study of the carbonation of CaO *when applied at the rate of 2 tons per acre, 8 inches, and immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED CaO
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2540	L1	5	No H_2O	11.95	0.0592	0.0673	0.0334	0.0339	678	1541	21.9
2566	L1	10	No H_2O	11.30	0.0618	0.0694	0.0336	0.0358	716	1627	23.1
2610	L1	19	No H_2O	10.75	0.0406	0.0455	0.0044	0.0411	822	1868	26.5
2636	L1	38	No H_2O	11.64	0.0188	0.0213	0.0134	0.0079	158	359	5.1
2662	L1	47	No H_2O	11.49	0.0430	0.0486	0.0243	0.0243	486	1104	15.8
2692	L1	61	No H_2O	10.54	0.0449	0.0502	0.0290	0.0290	424	964	13.7
2553	M1	5	H_2O added	13.90	0.0848	0.0985	0.0061	0.0924	1848	4200	59.9
2587	M1	10	H_2O added	14.30	0.0218	0.0254	0.0160	0.0094	188	427	6.1
2623	M1	19	H_2O added	12.53	0.0184	0.0210	0.0104	0.0106	212	482	6.8
2649	M1	38	H_2O added	14.05	0.0100	0.0116	0.0171	0.0000	000	000	0.0
2677	M1	47	H_2O added	14.50	0.0284	0.0321	0.0290	0.0031	62	141	2.0
2708	M1	61	H_2O added	14.65	0.0307	0.0360	0.0333	0.0027	54	123	1.7

native undisturbed red clay subsoil and it was intended that it should serve as a buffer, in case of any leaching during the period of experimentation. Each treatment was then mixed with the upper 6 inches of moist soil, equivalent to 150 pounds of moisture-free soil. The soil and treatments were mixed in metal lined boxes. Two men were assigned to each box and hoes were used to accomplish thorough mixing The treated soil was then placed in the cylinders and gently tamped.

At the beginning of the experiment it was thought well to consider the influence of varying soil moisture upon the rate of carbonation. Consequently, immediately after placing, one series was further moistened by the

addition of an inch of river water, applied evenly to the soil by the aid of a cheese-cloth covering. The other set was not further treated. During the 8-week period covered by the experiment, a total application of 8 inches of water supplemented the light natural rainfall and this was evenly distributed,

TABLE 12

A field-cylinder study of the carbonation of CaO *when applied at the rate of 2 tons per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED CaO
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2542	L7	5	12 T. manure, no H_2O	11.10	0.0688	0.0774	0.0334	0.0440	884	2000	28.4
2568	L7	10	12 T. manure, no H_2O	11.23	0.0588	0.0662	0.0336	0.0326	652	1482	21.3
2612	L7	19	12 T. manure, no H_2O	10.80	0.0343	0.0385	0.0044	0.0341	682	1550	22.0
2638	L7	38	12 T. manure, no H_2O	12.83	0.0220	0.0252	0.0134	0.0118	236	536	7.6
2665	L7	47	12 T. manure, no H_2O	12.23	0.0438	0.0499	0.0243	0.0256	512	1164	16.5
2695	L7	61	12 T. manure, no H_2O	10.51	0.0465	0.0520	0.0290	0.0230	460	1045	14.8
2555	M7	5	12 T. manure, and H_2O	14.80	0.0728	0.0854	0.0061	0.0793	1586	3604	51.1
2589	M7	10	12 T. manure, and H_2O	14.81	0.0267	0.0314	0.0160	0.0154	308	700	9.9
2625	M7	19	12 T. manure, and H_2O	12.00	0.0162	0.0184	0.0104	0.0080	160	364	5.1
2651	M7	38	12 T. manure, and H_2O	14.63	0.0162	0.0190	0.0171	0.0019	38	86	1.2
2680	M7	47	12 T. manure, and H_2O	15.66	0.0320	0.0379	0.0290	0.0089	178	404	5.7
2711	M7	61	12 T. manure, and H_2O	16.16	0.0375	0.0442	0.0333	0.0109	218	495	7.0

in order to prevent leaching. The treated soils were placed June 11, 1913, and were sampled at successive intervals of 5, 10, 19, 38, 47 and 61 days thereafter. The samples were collected for the full 8-inch depth to the clay subsoil and immediately bottled and transported to the laboratory where they were passed through a 2-mm. sieve, mixed and at once analyzed in the

moist condition for CO_2. The butter-sampler holes made in taking samples were immediately filled. The original soil was acid to litmus and was practically carbonate-free. The CO_2 obtained by its analysis might be considered on the whole as negligible and as well within laboratory error. With the moist unground acid soils, however, even after preliminary treatment for long periods with dilute HCl, there is always a slight CO_2 evolution due probably to action of acid upon soil organic matter or to residual traces of CO_2 condensed upon the surface of the soil particles. This small analytical

TABLE 13

A field-cylinder study of the carbonation of CaO *when applied at the rate of 2 tons per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED CaO
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2545	L13	5	30 T. manure, no H_2O	11.28	0.0504	0.0568	0.0355	0.0213	426	968	13.7
2574	L13	10	30 T. manure, no H_2O	11.30	0.0558	0.0629	0.0170	0.0457	914	207	28.1
2615	L13	19	30 T. manure, no H_2O	10.69	0.0408	0.0457	0.0041	0.0416	832	1891	26.8
2641	L13	38	30 T. manure, no H_2O	13.87	0.0339	0.0394	0.0258	0.0136	276	627	8.9
2668	L13	47	30 T. manure, no H_2O	13.90	0.0452	0.0525	0.0316	0.0209	418	950	13.5
2698	L13	61	30 T. manure, no H_2O	11.18	0.0446	0.0502	0.0316	0.0186	372	845	12.0
2558	M13	5	30 T. manure, and H_2O	14.70	0.1026	0.1203	0.0563	0.0640	1280	2909	41.3
2595	M13	10	30 T. manure, and H_2O	15.16	0.0226	0.0266	0.0039	0.0227	454	1032	14.6
2628	M13	19	30 T. manure, and H_2O	12.42	0.0194	0.0222	0.0020	0.0202	404	918	13.0
2654	M13	38	30 T. manure, and H_2O	15.78	0.0258	0.0306	0.0293	0.0013	26	59	0.8
2683	M13	47	30 T. manure, and H_2O	16.00	0.0364	0.0433	0.0261	0.0172	344	782	11.1
2714	M13	61	30 T. manure, and H_2O	15.94	0.0388	0.0462	0.0309	0.0153	306	695	9.9

error was, therefore, considered and blanks were run with each set of analyses. This point will be considered further by the consideration of experimental errors, as indicated by the data of table 22. In addition several attempts were made, without success, to determine any difference between the carbonate blank of untreated soil and that of the 6-ton manure treatments.

The increases of CO_2 resulting from carbonation of lime applied are given in tables 11 to 21, inclusive.

Discussion of speed of carbonation as determined from field cylinder studies

Two tons CaO *without water.* The lighter treatments of CaO without applications of water will be considered first.

Inspection of tables 11, 12 and 13 indicates a very slight but consistently greater apparent carbonate CO_2 content in the cylinders to which CaO was applied at the rate of 2 tons per acre, as compared to the cylinders receiving no lime. The small analytical differences, however, when converted into pounds per acre represent appreciable quantities, apparent or real The

TABLE 14

A field cylinder study of the carbonation of $Ca(OH)_2$ *when applied at the rate of 2 tons of* CaO *per acre 8 inches and immediately mixed throughout the upper 6 inches of the moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2541	L2	5	No H_2O	11.76	0.0760	0.0861	0.0334	0.0527	1054	2395	34.0
2567	L2	10	No H_2O	10.98	0.0618	0.0694	0.0336	0.0358	716	1627	23.1
2611	L2	19	No H_2O	10.44	0.0318	0.0355	0.0044	0.0311	622	1414	20.1
2637	L2	38	No H_2O	12.21	0.0225	0.0256	0.0134	0.0122	244	554	7.9
2663	L2	47	No H_2O	12.07	0.0325	0.0370	0.0243	0.0127	254	577	8.2
2693	L2	61	No H_2O	10.37	0.0379	0.0423	0.0290	0.0133	266	604	8.6
2554	M2	5	H_2O applied	14.20	0.0744	0.0867	0.0061	0.0806	1612	3664	52.0
2588	M2	10	H_2O applied	14.31	0.0264	0.0308	0.0160	0.0148	296	673	9.6
2624	M2	19	H_2O applied	11.92	0.0136	0.0154	0.0104	0.0050	100	227	3.2
2650	M2	38	H_2O applied	14.25	0.0268	0.0313	0.0171	0.0142	284	645	9.2
2678	M2	47	H_2O applied	15.32	0.0276	0.0326	0.0290	0.0036	72	164	2.3
2709	M2	61	H_2O applied	14.93	0.0344	0.0404	0.0333	0.0071	142	323	4.5

close agreement between the results of the first three samplings at the end of 5, 10 and 19 days, respectively, show that maximum carbonation ensued during the interval of 5 days between treatment and the first sampling. The carbonation of the lime not directly absorbed by the soil was apparently effected in large part by the initial CO_2 content of the moist soil. It is possible that the lime produced an acceleration in the generation of CO_2, thereby supplementing the initial supply available to effect carbonation. However, there appears to have been no acceleration in the rate of carbonation as a result of the simultaneous incorporation of dry organic matter, during the first three periods. This is not at all in harmony with what would

be anticipated from the results given by Fraps (18), who reported very extensive decompositions of various kinds of organic matter within 3 days after its contact with soil. However, in Fraps' studies 23 of the 25 soils studied were of sandy types and no caustic lime was added. The biological

TABLE 15

A field-cylinder study of the carbonation of $Ca(OH)_2$ *when applied at the rate of 2 tons per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2543	L8	5	12 T. manure, no H_2O	11.83	0.0808	0.0916	0.0334	0.0682	1364	3100	44.0
2569	L8	10	12 T. manure, no H_2O	10.84	0.0540	0.0606	0.0336	0.0270	540	1227	17.4
2613	L8	19	12 T. manure, no H_2O	10.69	0.0284	0.0318	0.0044	0.0274	548	1245	17.7
2639	L8	38	12 T. manure, no H_2O	11.18	0.0202	0.0227	0.0134	0.0093	186	423	6.0
2666	L8	47	12 T. manure, no H_2O	12.14	0.0388	0.0442	0.0243	0.0199	398	904	12.8
2696	L8	61	12 T. manure, no H_2O	10.50	0.0432	0.0483	0.0290	0.0193	386	877	12.4
2556	M8	5	12 T. manure, and H_2O	14.70	0.0892	0.1045	0.0061	0.0984	1968	4454	63.2
2590	M8	10	12 T. manure, and H_2O	14.58	0.0271	0.0317	0.0160	0.0157	314	714	10.1
2626	M8	19	12 T. manure, and H_2O	11.72	0.0186	0.0211	0.0104	0.0107	214	486	6.9
2652	M8	38	12 T. manure, and H_2O	15.25	0.0144	0.0170	0.0171	0.0000	000	000	0.0
2681	M8	47	12 T. manure, and H_2O	15.60	0.0354	0.0419	0.0290	0.0129	258	586	8.3
2712	M8	61	12 T. manure, and H_2O	15.52	0.0380	0.0450	0.0333	0.0117	234	554	7.8

studies of Hutchinson (37, 38) would suggest the probability of a marked acceleration of bacterial activities and an absence of partial sterilizing effect from this light treatment of lime The analyses of the same cylinders at the latter three periods of contact, 38, 47 and 61 days, shows a consistent decrease

in the amount of $CaCO_3$. These data point to direct absorption by the soil of a considerable part of the CaO followed by a carbonation of the fraction not immediately absorbed. Further absorption by the soil of that portion of the lime which underwent carbonation then ensued. It is possible, how-

TABLE 16

A field-cylinder study of the carbonation of $Ca(OH)_2$ *when applied at the rate of 2 tons of* CaO *per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2546	L14	5	30 T. manure, no H_2O	11.70	0.0506	0.0573	0.0355	0.0218	436	991	12.6
2575	L14	10	30 T. manure, no H_2O	10.98	0.0398	0.0447	0.0170	0.0277	554	1282	18.2
2616	L14	19	30 T. manure, no H_2O	10.62	0.0300	0.0336	0.0041	0.0295	590	1341	19.0
2642	L14	38	30 T. manure, no H_2O	14.50	0.0341	0.0399	0.0258	0.0141	282	641	9.1
2669	L14	47	30 T. manure, no H_2O	13.54	0.0546	0.0632	0.0316	0.0316	732	1664	23.6
2699	L14	61	30 T. manure, no H_2O	11.63	0.0430	0.0487	0.0316	0.0171	342	777	11.0
2559	M14	5	30 T. manure, and H_2O	14.60	0.0546	0.0639	0.0563	0.0076	152	345	4.9
2596	M14	10	30 T. manure, and H_2O	15.30	0.0176	0.0208	0.0039	0.0169	338	768	10.9
2629	M14	19	30 T. manure, and H_2O	12.30	0.0162	0.0185	0.0020	0.0165	330	750	10.6
2655	M14	38	30 T. manure, and H_2O	13.52	0.0322	0.0372	0.0293	0.0079	158	359	5.9
2684	M14	47	30 T. manure, and H_2O	10.05	0.0411	0.0457	0.0261	0.0196	392	891	12.6
2715	M14	61	30 T. manure, and H_2O	16.10	0.0372	0.0443	0.0309	0.0134	268	509	7.2

ever, that carbonation preceded absorption, i.e., that the lime absorbed by the soil during the first interval of 5 days may have been derived largely from the form of $CaCO_3$, to which compound the CaO may have been converted subsequent to its hydration and solution by the soil moisture. It seems

definitely established, however, that maximum carbonation ensued within 5 days, whereas the maximum of measurable absorption did not occur until between 10 to 19 days after treatment. In the summation of the data from the twelve 2-ton cylinders, this fact will be further discussed.

Two tons of CaO *plus water.* Considering next the cylinders treated in like manner as to amount of CaO and manure, but with additions of water, the data of tables 11, 12 and 13 show a marked variation in the rate of carbonation effected. In each case the maximum carbonation is indicated at the end of the first interval of 5 days. Beyond that period, the decrease in car-

TABLE 17

A field-cylinder study of the carbonation of CaO *when applied at the rate of 8 tons per acre 8 inches and immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED CaO
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2551	L25	5	No H_2O	11.19	0.1280	0.1441	0.0334	0.1107	2214	5,032	17.9
2585	L25	10	No H_2O	7.37	0.3184	0.3437	0.0336	0.3101	6202	14,095	50.0
2621	L25	19	No H_2O	10.33	0.3678	0.4102	0.0044	0.4058	8116	18,432	65.4
2647	L25	38	No H_2O	11.60	0.3814	0.4315	0.0134	0.4181	8362	19,004	67.4
2674	L25	47	No H_2O	11.56	0.3812	0.4310	0.0243	0.4067	8134	18,486	65.6
2704	L25	61	No H_2O	10.38	0.3922	0.4376	0.0290	0.4086	8172	18,572	65.9
2564	M25	5	H_2O applied	14.60	0.1863	0.2182	0.0266	0.1916	3832	8,709	30.9
2606	M25	10	H_2O applied	12.39	0.2443	0.2789	0.0160	0.2629	5258	11,950	42.4
2634	M25	19	H_2O applied	12.68	0.2316	0.2652	0.0104	0.2548	5096	11,582	41.1
2660	M25	38	H_2O applied	15.70	0.3530	0.4188	0.0171	0.4017	8034	18,259	64.8
2689	M25	47	H_2O applied	15.53	0.3722	0.4404	0.0290	0.4114	8228	18,700	66.4
2720	M25	61	H_2O applied	16.63	0.3892	0.4612	0.0333	0.4279	8558	19,450	69.0

bonates is very marked. It is possible that the water added resulted in a marked and immediate increase in biological activities, thus causing a speedy carbonation followed by more thorough diffusion of dissolved $CaCO_3$ and hence greater absorption. In these cylinders it appears that maximum carbonation and minimum absorption occurred at a much earlier period than the 10 to 19 days' interval required by the unwetted cylinders.

The data derived from the six wetted cylinders demonstrate beyond doubt that complete carbonation ensued uniformly within the period of 5 days. In these cylinders the abundance of moisture available to permeate

the very fine lime particles very probably prevented the protective action of $CaCO_3$ crusts and the attaining of apparent equilibrium before complete carbonation was actually effected, differing in this manner from the case of dry mulch results of table 10.

TABLE 18

A field-cylinder study of the carbonation of CaO *when applied at the rate of 8 tons of* CaO *per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cen*
2548	L19	5	48 T. manure, no H_2O	11.29	0.1408	0.1587	0.0107	0.1480	2960	6,727	23.9
2580	L19	10	48 T. manure, no H_2O	10.71	0.2136	0.2392	0.0187	0.2205	4410	10,023	35.5
2618	L19	19	48 T. manure, no H_2O	10.49	0.3264	0.3647	0.0075	0.3572	7144	14,288	56.7
2644	L19	38	48 T. manure, no H_2O	12.62	0.4170	0.4772	0.0264	0.4508	9016	18,032	64.0
2671	L19	47	48 T. manure, no H_2O	12.67	0.3952	0.4525	0.0313	0.4212	8424	16,848	59.8
2701	L19	61	48 T. manure, no H_2O	10.69	0.4076	0.4564	0.0318	0.4246	8492	16,984	60.3
2561	M19	5	48 T. manure, and H_2O	14.40	0.1584	0.1850	0.0310	0.1540	3080	6,160	21.9
2601	M19	10	48 T. manure, and H_2O	15.32	0.2150	0.2539	0.0135	0.2404	4808	9,616	34.1
2631	M19	19	48 T. manure, and H_2O	12.72	0.3670	0.4205	0.0038	0.4167	8334	16,668	59.1
2657	M19	38	48 T. manure, and H_2O	16.47	0.3792	0.4540	0.0286	0.4254	8508	17,016	60.4
2686	M19	47	48 T. manure, and H_2O	16.03	0.3982	0.4741	0.0276	0.4465	8930	17,860	63.4
2717	M19	61	48 T. manure, and H_2O	15.98	0.3730	0.4440	0.0325	0.4115	8230	16,460	58.4

Two tons of CaO *as* $Ca(OH)_2$, *without water.* Coming next to the consideration of 2-ton treatments of CaO, when applied as $Ca(OH)_2$ without addition of water, we find no marked difference between the activities of the hydrate, as compared to the oxide. This would be expected for the CaO would

quickly combine with soil moisture giving $Ca(OH)_2$ and in effect, producing identical treatments. Cylinders 12 and 18 show, as do their corresponding cylinders receiving CaO, that maximum carbonation ensued within 5 days while the difference indicated by the analysis as occurring in cylinder 14 between the 5- and 10-day interval is hardly sufficient to justify the statement that, in this case, maximum carbonation was delayed until the 10-day interval. As a matter of fact, however, the corresponding cylinders L13 and L14 exhibit somewhat the same tendency, if the small analytical differences are considered as sufficiently positive to register such retardation.

TABLE 19

A field-cylinder study of the carbonation of $Ca(OH)_2$ *when applied at the rate of 8 tons of* CaO *per acre 8 inches and immediately mixed throughout the upper 6 inches of a moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*	*per cent*
2552	L26	5	No H_2O	11.20	0.1376	0.1550	0.0334	0.1216	2432	5,527	19.5
2585	L26	10	No H_2O	8.22	0.2626	0.2861	0.0336	0.2525	5050	11,477	40.7
2622	L26	19	No H_2O	9.85	0.2614	0.2900	0.0044	0.2856	5712	12,982	46.1
2648	L26	38	No H_2O	11.87	0.3754	0.4259	0.0134	0.4125	8250	18,704	66.3
2675	L26	47	No H_2O	8.47	0.3900	0.4261	0.0243	0.4018	8036	18,241	64.7
2705	L26	61	No H_2O	12.23	0.3844	0.4380	0.0290	0.4090	8180	18,591	66.0
2565	M26	5	H_2O applied	15.00	0.1782	0.2096	0.0061	0.2035	4070	9,250	32.8
2606	M26	10	H_2O applied	10.94	0.3026	0.3360	0.0160	0.3200	6400	14,545	51.5
2635	M26	19	H_2O applied	12.92	0.2504	0.2876	0.0104	0.2772	5544	12,600	44.7
2661	M26	38	H_2O applied	16.22	0.3594	0.4290	0.0171	0.4119	8238	18,704	67.1
2690	M26	47	H_2O applied	15.54	0.3858	0.4568	0.0290	0.4278	8556	19,432	68.9
2721	M26	61	H_2O applied	15.72	0.3722	0.4415	0.0333	0.4082	8164	18,554	65.8

Two tons of CaO, *as* $Ca(OH)_2$, *plus water.* Duplication of the above treatments was also effected with additions of water. From the data of tables 14, 15 and 16 it appears that the cylinders receiving no manure and the smaller applications of manure attained maximum carbonation within 5 days, as did their corresponding CaO-treated cylinders, while maximum immediate absorption had been practically effected by the end of the 10-day period. However, where the heavy manure treatment was applied, maximum absorption and maximum carbonation seemed to be coincident.

It should be borne in mind that the analyses of cylinders were carried out upon moist soils. These soils were analyzed the same day as sampled, first

being run through a 2-mm. sieve and mixed. In this way, the soil moisture was devoid of increased pressure and its structure was so disintegrated as to induce diffusion of soil atmosphere to the air. No differences could be detected between the CO_2 liberated by acid from the moist check soils thus

TABLE 20

A field-cylinder study of the carbonation of $Ca(OH)_2$ *when applied at the rate of 8 tons of* CaO *per acre 8 inches together with dry barnyard manure and both substances immediately mixed throughout the upper 6 inches of moist acid Cumberland loam soil*

LABORATORY NUMBER	CYLINDER NUMBER	NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	TREATMENT OTHER THAN LIME	MOISTURE AT TIME OF SAMPLING	CO_2 IN WET SOIL	CO_2 IN MOISTURE-FREE SOIL	CO_2 IN BLANK MOISTURE-FREE SOIL	INCREASE IN CO_2 IN MOISTURE-FREE SOIL FROM TREATMENT	INCREASE PER ACRE 2,000,000 POUNDS OF SOIL CALCULATED AS		INCREASE OF $CaCO_3$ PER CENT OF THEORY FROM APPLIED $Ca(OH)_2$
									CO_2	$CaCO_3$	
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs*	*per cent*
2549	L20	5	48 T. manure, no H_2O	11.21	0.1416	0.1595	0.0107	0.1488	2976	6,764	24.0
2581	L20	10	48 T. manure, no H_2O	11.42	0.2010	0.2271	0.0187	0.2084	4168	9,473	33.6
2619	L20	19	48 T. manure, no H_2O	10.27	0.3266	0.3640	0.0075	0.3565	7130	16,204	57.5
2645	L20	38	48 T. manure, no H_2O	12.30	0.4326	0.4933	0.0264	0.4669	9338	21,223	75.3
2672	L20	47	48 T. manure, no H_2O	11.56	0.4074	0.4607	0.0313	0.4294	8588	19,518	65.7
2702	L20	61	48 T. manure, no H_2O	12.00	0.4002	0.4547	0.0318	0.4229	8458	19,277	68.4
2562	M20	5	48 T. manure, and H_2O	14.50	0.1816	0.2124	0.0310	0.1814	3628	8,245	29.3
2602	M20	10	48 T. manure, and H_2O	15.27	0.2150	0.2538	0.0135	0.2383	4766	10,363	36.7
2632	M20	19	48 T. manure, and H_2O	13.46	0.3552	0.4105	0.0038	0.4067	8134	18,486	65.6
2658	M20	38	48 T. manure, and H_2O	16.27	0.3980	0.4754	0.0286	0.4468	8936	20,309	72.1
2687	M20	47	48 T. manure, and H_2O	14.13	0.4058	0.4726	0.0276	0.4450	8900	20,227	71.8
2718	M20	61	48 T. manure, and H_2O	16.18	0.3944	0.4706	0.0325	0.4381	8762	19,914	70.6

treated and that obtained from the same soils after air-drying, by most careful manipulation. Theoretically, however, it might be assumed that in the fresh soil, part of the determined CO_2 was to be attributed to possible gaseous CO_2 from accelerated biological activities, in case all of the lime

had been carbonated. Comparisons between the checks of precipitated $CaCO_3$ and the untreated soil, however, permitted no such assumption.

A summation of the analyses from the twelve cylinders, to which the 2-ton treatments were applied, points conclusively to one fact, namely, that not all of the oxide and hydrate treatments were absorbed as such, prior to carbonation. Furthermore, the oxide and hydrate treatments reached their maximum carbonation within 5 days and then followed absorption of lime from the carbonate. During the succeeding 5 days there was effectuated maximum immediate absorption from the lighter treatment. This is borne out by the following data. The average of 24 determinations upon the twelve cylinders which received the same amount of CaO as burnt oxide or hydrate gave 0.0450 per cent CO_2 at the end of 61 days of contact between soil and treatment; while the average of 8 determinations upon the precipitated $CaCO_3$ checks gave 0.0437 per cent CO_2. But, the average of 24 determinations from the twelve cylinders at the end of 5 days of contact between lime and soil gave an occurrence of 0.0830 per cent CO_2. During the succeeding 5 days, however, there occurred a decrease in the CO_2 content of these twelve cylinders to 0.0449 per cent, as an average from 24 determinations. Thus for 5, 10 and 61 days of contact, the CO_2 occurrences indicated by the analyses were 0.0830 per cent, 0.0449 per cent and 0.0450 per cent, respectively, from a treatment of 2 tons per acre of CaO, as compared to 0.0437 per cent for the final residual CO_2 content from the equivalent treatments of precipitated $CaCO_3$.

Eight-ton treatments. The 8-ton treatments in the cylinders furnished considerable excess of lime above the immediate absorption coefficient. They thus afforded better opportunity for chemical analysis and a more satisfactory basis of comparison than was possible in the case of the 2-ton treatments. The analyses of the 8-ton treatments are given in tables 17 to 21, inclusive. As a whole, the soils of the 8-ton treatments may be said to have effected complete carbonation during the period extending between 19 and 38 days after treatment. No difference could be noted between the rate of carbonation in moist soil, without supplementary rainfall and the soil which received water, except possibly during the first period of 15 days. Nor could there be observed any difference between the activities of the oxide and hydrate forms. In comparing the treatments of burnt and hydrated lime when applied alone, and when supplemented by dry barnyard manure no acceleration of carbonate formation could be attributed to the action of the barnyard manure. As differing from the cylinders receiving the lighter applications, it is quite possible that partial retardation of biological activities may have resulted from the 8-ton treatments. This would influence CO_2 evolutions, both in regard to that derived from decomposition of initial organic matter and that derived from the supplementary treatments of barnyard manure. Hutchinson (37) demonstrated a distinct sterilizing effect from treatments of CaO above 0.5 per cent. However, the soils used by

TABLE 21

Comparison between total amounts of $CaCO_3$ *residual from chemically equivalent amounts of* CaO, $Ca(OH)_2$ *and "c.p." precipitated* $CaCO_3$ *when in excess of lime requirements, after contact between treatment and moist acid Cumberland loam soil in field-cylinders*

Basis of treatment 8 tons CaO *per 2,000,000 pounds of soil*

LABORATORY NUMBER	CYLINDER NUMBER	TIME OF CONTACT	TREATMENT	MOISTURE AT TIME OF SAMPLING	CO_2 MOIST SOIL	CO_2 MOISTURE-FREE SOIL	$CaCO_3$ MOISTURE-FREE SOIL	AVERAGE $CaCO_3$ MOISTURE-FREE SOIL		AVERAGE RESIDUAL OF $CaCO_3$ FROM CaO AND $Ca(OH)_2$, AS COMPARED WITH THAT RESIDUAL FROM $CaCO_3$ EQUIVALENT TREATMENT
		days		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*
2674	L25	47	CaO, no H_2O applied	11.58	0.3812	0.4310	0.980			
2675	L26	47	$Ca(OH)_2$, no H_2O applied	8.47	0.3900	0.4261	0.970			
2671	L19	47	CaO and 48T. manure, no H_2O	12.67	0.3952	0.4525	1.028	Eight oxide and hydrate cylinders	1.027	96.1
2672	L20	47	$Ca(OH)_2$, 48 T. manure, no H_2O	11.56	0.4074	0.4607	1.047			
2676	L27	47	$CaCO_3$, no H_2O applied	12.81	0.4212	0.4831	1.098			
2689	M25	47	CaO, water applied	15.53	0.3722	0.4404	1.001			
2690	M26	47	$Ca(OH)_2$ water applied	15.54	0.3858	0.4568	1.038			
2686	M19	47	CaO and 48 T. manure, and H_2O	16.03	0.3982	0.4741	1.077	Two checks	1.069	
2687	M20	47	$Ca(OH)_2$ and 48 T. manure, and H_2O	14.13	0.4058	0.4726	1.074			
2691	M27	47	$CaCO_3$, water applied	15.40	0.3871	0.4576	1.040	Difference	0.042	

2704	L25	61	CaO, no H_2O applied	10.38	0.3922	0.4376	0.994	Eight oxide and hydrate cylinders	1.024	96.6
2705	L26	61	$Ca(OH)_2$, no H_2O applied	12.23	0.3844	0.4377	0.994			
2701	L19	61	CaO and 48 T. manure, no H_2O	10.69	0.4076	0.4564	1.037			
2702	L20	61	$Ca(OH)_2$, and 48 T. manure, no H_2O	12.00	0.4002	0.4547	1.033			
2706	L27	61	$CaCO_3$, no water applied	12.06	0.4012	0.4562	1.037			
2720	M25	61	CaO, water applied	16.63	0.3892	0.4612	1.048	Two checks	1.060	
2721	M26	61	$Ca(OH)_2$, water applied	15.72	0.3722	0.4415	1.003			
2717	M19	61	CaO, 48 T. manure, and H_2O	15.98	0.3730	0.4440	1.009			
2708	M20	61	$Ca(OH)_2$, 48 T. manure, and H_2O	16.18	0.3944	0.4706	1.070			
2722	M27	61	$CaCO_3$, water applied	15.97	0.4006	0.4768	1.083	Difference	0.036	

Hutchinson were initially calcareous and hence absorbed little if any of the hydrate; while the cylinder soil was distinctly acid before treatment, and consequently the process of absorption quickly removed a considerable part of the applied lime from the active hydrate form.

Considering the possibility of errors involved in sampling and analysis, it may be said that the occurrence of carbonates was constant after 38 days of contact between lime and soil. The analyses of check treatments of precipitated $CaCO_3$ at the end of 47 and 61 days showed that the check treatments had also reached a state of apparent equilibrium.

In table 21 are given the percentages of carbonate produced by the carbonation of caustic lime and the amount of residual carbonates from the check treatments of precipitated $CaCO_3$, at the end of the longest two intervals between placing and sampling. The average of the eight oxide and hydrate treatments are 0.042 and 0.036 per cent less than the average of the two $CaCO_3$ applications for the 47- and 61-day periods, respectively. The variations between the two checks at each interval are greater than the differences above given as occurring between the soils receiving the two forms of lime. If instead of two checks, eight checks were available to average against 8 cylinders receiving oxide and hydrate treatments, the slight discrepancy might be eliminated. It might be argued, however, that the difference is real. It could be held that the active mass of dissociated calcium ions was greater in the hydrate than in the carbonate and that considerable part of the more strongly dissociated hydrate was in contact with the soil for a number of days prior to carbonation. This would quite probably result in greater absorption of calcium from the hydrate treatment than from a chemically equivalent amount of the less soluble $CaCO_3$. Again, it might be maintained that where no water was added small lumps of lime were carbonated only on the surface of the lumps, and that this carbonate layer would protect enclosed portions of uncarbonated hydrate. However, in view of the thorough dissemination effected and the extreme fineness of the lime and also the chance for absorption of carbonated soil water, such a contention would seem altogether untenable. Furthermore, it might be contended that a part of the lime acted upon or combined with organic matter in such a way as to decrease the amount of lime available for direct carbonation. However, if it be granted that the assumed chemical action of lime upon organic matter is such as to cause a generation of CO_2, the above contention would be invalidated; for it will be shown, by the data of tables 54 and 55, that no such gaseous generation is brought about.

Again, it might be held that the flocculation produced by the uncarbonated lime would result in a physical occlusion of part of the lime that would result in the removal of the lime so occluded from the sphere of chemical activity. Such a viewpoint could not well be disproved. As a matter of fact, it will be shown in the lysimeter studies that after treatment with burnt lime we are unable to obtain at any period, amounts of $CaCO_3$ equivalent to the car-

bonate residual from chemically equivalent amounts of the precipitated carbonate used as a check. And, since the difference between the amounts of lime leached from the two forms and the differences in amounts used in neutralizing acids engendered by nitrification and sulfofication would not account for the deficiency of the carbonate resulting from applications of burnt lime, it is necessary to assume such a physical occlusion or else a greater absorption of lime from the solution phase of $Ca(OH)_2$. The latter would seem to be more in consonance with the chemistry of the problem.

TABLE 22

Showing periodical variations of carbonate-blank CO_2, either actually occurring in cylinder blanks receiving manure alone, or errors introduced in sampling and analysis

NUMBER OF DAYS BETWEEN TREATMENT AND SAMPLING	SOIL APPLICATIONS OF DRY BARNYARD MANURE, AT RATES PER 2,000,000 POUNDS OF MOISTURE-FREE SOIL					
	24,000 lbs.		60,000 lbs.		96,000 lbs.	
	CO_2 in moisture-free soil		CO_2 in moisture-free soil		CO_2 in moisture-free soil	
	No water applied	Water applied	No water applied	Water applied	No water applied	Water applied
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5	0.0334	0.0061	0.0355	0.0563	0.0107	
10	0.0336	0.0160	0.0170	0.0039	0.0187	0.0135
19	0.0044	0.0211	0.0041	0.0020	0.0075	0.0038
38	0.0134	0.0171	0.0258	0.0293	0.0264	0.0286
47	0.0243	0.0290	0.0316	0.0261	0.0313	0.0276
61	0.0290	0.0333	0.0316	0.0309	0.0318	0.0325
Variations, minimum to maximum.....	0.0298	0.0272	0.0314	0.0543	0.0233	0.0287

Consideration of analytical error involved in the determination of carbonates in the cylinders

By reference to table 22, the reader will see the differences which may be expected in the determination of the meagre amounts of CO_2 in that series of checks which have been treated with manure without lime. The deviations and fluctuations may be considered as real, or as being due to error introduced through sampling or through chemical manipulation. In only one instance is the variation between maximum and minimum of the magnitude of the difference found between the CaO and $Ca(OH)_2$ treatments and the $CaCO_3$ checks. Hence, it might be assumed, within reason, that a part of the determined deficiency of $CaCO_3$ below that of theory resulting from the CaO and $Ca(OH)_2$ treatments may be actually attributable to the several possible limiting factors discussed in the preceding paragraph. The amounts of $CaCO_3$ derived from the residual CaO and $Ca(OH)_2$, not absorbed directly by the soil are consistently, though not materially, less than those remaining

from the equivalent applications of $CaCO_3$. However, without supplementary data, the differences are not of such magnitude as to justify the unqualified statement that the two caustic forms have been more active than the precipitated carbonate in the neutralization of acid soils. But, when fortified by the residual carbonate and leaching data obtained later in the 8-ton CaO lysimeter treatments, it would seem conservative to conclude that more lime has been absorbed by the soil from the oxide and hydrate mixed throughout the soil, than from equivalent amounts of precipitated $CaCO_3$ incorporated in the soil simultaneously and in the same manner. Again, further data obtained in other analyses of the same series of cylinders demonstrated the fact that more lime was absorbed by the soil from treatments of precipitated $CaCO_3$ than from equivalent and simultaneous treatments of ground limestone. Since the precipitated $CaCO_3$ was more soluble than the limestone, this would be expected and it is in accord with the differences manifested between the oxide, or hydrate, and precipitated carbonate. It would appear then, that in a given time the extent of absorption, fixation or silication by the soil of equivalent and simultaneous applications of calcareous materials would vary with the material applied. Burnt and hydrated lime seem identical and are first in order followed by precipitated carbonate, and it in turn by limestone.

POT STUDIES IN THE FIELD

In the foregoing field cylinder studies, the carbonation problem represented but one phase of the problem as a whole. In the more comprehensive problem which embraced the carbonation studies it was necessary that all of the treatments applied should be worked thoroughly throughout the entire volume of soil, in order to justify the sampling which was planned for the other subsequent work that was to extend over a long period. Hence, in the cylinder carbonation studies, the mixing of lime with the acid soil involved a conflict of the carbonation and absorption factors which has been discussed in preceding paragraphs. The lightest cylinder treatment mixed with soil was based upon a 4000-pound application for each 2,000,000 pounds of moisture-free soil. It seemed desirable, therefore, to attempt a study of the carbonation of a treatment equivalent to the more common application of 2000 pounds per acre surface. The cylinder experiments with but one loam soil, previously discussed, demonstrate that such an application would have been so speedily absorbed by the acid soil as to make impossible a reliable quantitative study of the formation of $CaCO_3$. It was necessary, therefore, to eliminate or minimize this factor of absorption. This was done by subjecting seven acid soils to a preliminary treatment with precipitated $CaCO_3$. Such a preliminary treatment would so vitiate the normal condition of the acid soil as to make it imperative that time be allowed for a return to normality before application of $Ca(OH)_2$. In addition, it appeared advisable

to consider the possible influence of the reaction of the soil immediately underlying that with which the lime was to be mixed; this, because of the possible differential introduced by variation of soil reaction in influencing the extent of upward diffusion of CO_2 from the underlying soil to that overlying and mixed with $Ca(OH)_2$; as well as the contention that so-called acids other than H_2CO_3 will diffuse, upward from an acid soil. Accordingly ten pots were filled with each of seven soils. Each pot was given its respective preliminary and experimental treatment as outlined in table 23. Thirty-five of the pots were used to contain seven checks and 28 treatments which were to be sampled at the end of 4 days, and the same number were to be used for a second sampling, which was to be made at a time indicated as desirable by

TABLE 23

Weights of soils in pots (total and by zones); amounts of $CaCO_3$ *used in preliminary treatments and amounts of* $Ca(OH)_2$ *applied*

SOILS USED	WET SOIL PER ENTIRE POT	WET SOIL PER UPPER ZONE	WET SOIL PER LOWER ZONE	MOISTURE-FREE SOIL PER ENTIRE POT	MOISTURE-FREE SOIL PER UPPER ZONE	MOISTURE-FREE SOIL PER LOWER ZONE	$CaCO_3$ PER ENTIRE POT	$CaCO_3$ PER UPPER ZONE	$CaCO_3$ PER LOWER ZONE	$Ca(OH)_2$ SURFACE AND MIXED THROUGHOUT UPPER ZONE = 2000 POUNDS CaO PER 2,000,000 POUNDS OF SOIL
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Jackson sandy loam	2214	1377	837	2162	1345	817	10.8010	6.7175	4.0835	1.7950
Cherokee sandy loam	2066	1350	716	1914	1251	663	9.5695	6.2545	3.3150	1.6701
Dolomitic gravelly loam	2417	1345	1072	2226	1239	987	11.1315	6.1945	4.9370	1.6541
Indian mound loam	2324	1377	947	2093	1240	853	10.4630	6.1995	4.2635	1.6554
Second bench loam	2276	1389	887	1997	1219	778	9.9840	6.0930	3.8910	1.6269
Chickamauga loam	2389	1425	964	2005	1196	809	10.0245	5.9795	4.0450	1.5967
Tellico sandy loam	2044	1184	860	1783	1033	750	8.9150	5.1640	3.7510	1.3789

$CaCO_3$ treatments based upon treatment of 10,000 pounds of carbonate per 2,000,000 pounds of moisture-free soil.

the results secured from the first sampling. The details of the experiment were as follows: The clay pots used were 8 inches in depth and 7 inches in diameter. They were painted on the outside with black asphaltum paint, except on the bottom, which were left unpainted. The outlets for drainage were unstoppered. Immediately after the preliminary $CaCO_3$ treatment, the pots were imbedded in the soil outdoors, where they were left exposed for a period of 14 months. The $CaCO_3$ treatments were made at the rate of 10,000 pounds of $CaCO_3$ per 2,000,000 pounds of moisture-free soil. In one-half of the number of pots used the entire 6 inches of soil was treated with $CaCO_3$ at this rate. In the remaining pots, the upper 3-inch zone received the preliminary $CaCO_3$ treatment, but the lower zone was unaltered,

TABLE

Comparison between the reversion of hydrated lime to carbonate, when exposed on the surface and when mixed within the upper 3 inches of soils; embedded pot containers; treatments equivalent to CaO *at the rate of 2,000 pounds per 2,000,000 pounds of soil*

SOIL	WHERE ENTIRE 6 INCHES OF SOIL WERE GIVEN AN APPLICATION OF $CaCO_3$ AT THE RATE OF 10,000 POUNDS PER 2,000,000 POUNDS OF SOIL 14 MONTHS PRIOR TO APPLICATION OF $Ca(OH)_2$									WHERE UPPER 3 INCHES ONLY WERE TREATED WITH $CaCO_3$ AT THE RATE OF 10,000 POUNDS PER 2,000,000 POUNDS OF SOIL 14 MONTHS PRIOR TO APPLICATION OF $Ca(OH)_2$								
	Blank	Increase in CO_2 content of soils over blanks after applications of $Ca(OH)_2$								Blank	Increase in CO_2 content of soils over blanks after applications of $Ca(OH)_2$							
		Applied to surface				Mixed with 3 inches of soil					Applied to surface				Mixed with 3 inches of soil			
		After 4 days		After 10 days		After 4 days		After 10 days			After 4 days		After 10 days		After 4 days		After 10 days	
		As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation		As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation	As CO_2 in soil	As per cent carbonation
	gm.	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
Jackson sandy loam	0.048	0.091	115	0.072	91	0.068	86	0.066	84	0.045	0.056	71	0.070	89	0.056	71	0.070	89
Cherokee sandy loam	0.015	0.080	101	0.019	25	0.033	42	0.042	53	0.014	0.050	63	0.050	63	0.036	46	0.042	53
Dolomitic gravelly loam	0.155	0.110	139	0.078	99	0.096	122	0.085	108	0.173	0.092	116	0.036	46	0.041	52	0.049	62
Indian mound loam	0.020	0.086	109	0.058	73	0.037	47	0.036	46	0.011	0.085	108	0.049	62	0.042	53	0.047	60
Second river bench loam	0.046	0.064	81	0.074	94	0.059	75	0.058	73	0.031	0.083	105	0.077	97	0.066	84	0.059	75
Chicamauga loam	0.101	0.086	109	0.073	92	0.073	92	0.069	87	0.093	0.118	149	0.084	106	0.065	82	0.065	82
Tellico sandy loam	0.011	0.042	53	0.030	38	0.021	27	0.029	37	0.011	0.037	47	0.044	56	0.029	37	0.024	30
Averages	0.057	0.080	108	0.058	75	0.055	70	0.055	70	0.056	0.074	94	0.059	74	0.048	61	0.051	64

save for sifting and mixing. Each upper and lower 3-inch zone was separated by a galvanized iron wire disc made from $\frac{1}{8}$-inch wire cloth. To each disc was attached galvanized wire handles, which permitted the removal of the upper zone *en bloc*, without disturbing the lower zone. At the end of the 14 months' exposure, the lime treatments were applied. Hydrated lime, 99.86 per cent pure, was mixed through the entire upper 3-inch zone of one-half the number of pots, while the remaining pots received the lime on the soil surfaces. The upper zones of the check pots were removed, mixed and replaced in the same manner as were those of the pots receiving $Ca(OH)_2$. The 14 months' exposure would seem to represent a reasonable period, during which the $CaCO_3$-treated soils would be expected to return to a nearly normal condition; however, such a period also afforded an opportunity for the relatively soluble precipitated carbonate to leach out and leave the soil with a further tendency to absorb additional quantities of lime. Four days after the application of hydrated lime one-half of the total number of pots was conveyed to the laboratory and dried in electric ovens at 105°C. The remaining pots were sampled and analyzed at the end of 10 days of exposure. The data secured in this manner are incorporated in table 24. The relative meagreness of the amount of hydrate applied and the difficulties of technic are responsible for limitations which may well be considered.

Discussion of the data of table 24

The data of this table serve as a good illustration of the difficulty encountered in recording chemical changes resulting from soil treatments in amounts which would be considered as within the limits of good practice. An increase of 0.079 per cent represents the theoretical increase of CO_2 which would be possible from the lime applied at the 1-ton rate. A relatively small percentage of error, therefore, represents a considerable proportion of the total of the amount representing the possible increase.

In addition, certain insuperable but essential conditions of control tended to vitiate the results. In working with so many samples at the same time it was necessary to heat simultaneously, immediately after their removal from the pots in the field, in order to eliminate moisture and inhibit any further generation of CO_2. This would be expected to augment the amount of lime absorbed by the soil in the case of the mixtures of lime throughout the soil, while it would cause but slight, if any, absorption in the case of the surface treatments, which were in contact with but a fraction of the whole amount of soil.

Again, the simultaneous heating of the contents of each of the 35 pots, of each period of exposure, in two large electric ovens, occasioned the giving-off of the CO_2 content of the soil moisture, and possibly some of that condensed upon surfaces of the soil particles. Any residual hydrate of that internally or externally applied would thus have access to moist atmospheric CO_2.

Considering the averages obtained from the seven soils, it appears that there was no enhancement in the gain of $CaCO_3$ at the end of 10 days, as compared to that found at the end of 4 days; this applying to both surface and intra-soil treatments. Nor does it appear that any difference was registered between the soil underlaid with 3 inches of acid soil as compared to that which was underlaid with 3 inches of alkaline soil. Furthermore, recognizing the limitations above referred to, it would seem that one of two conclusions is possible. Either all of the lime mixed within and applied to the surface at the rate of 1 ton per 2,000,000 pounds of soil, had reverted to the carbonate at the end of 4 days, or any residual hydrate occurring at the time of sampling was converted to the carbonate by the CO_2 given off in the ovens during the process of drying. It is possible, however, that the CO_2 derived from field exposure and from oven-drying brought about the maximum carbonation to be effected with lime of the fineness of that exposed: but, had there been induced a disruption of the pseudo-equilibrium through wetting or grinding, there might have occurred a greater average in the gain of carbonate CO_2.

It is evident from the foregoing that in order to secure data of positive nature and value it is essential that a heavier rate of application, fewer soils, and smaller amounts be employed, under conditions permitting better contact and speedier manipulation.

Accordingly, further experiments were planned, which are reported under the designation of "Tumbler experiments."

CARBONATION STUDIES IN FIELD LYSIMETER TANKS

The study of the carbonation resulting from exposure of burnt and hydrated lime in the originally acid Cumberland loam soil of the cylinders was continued by the installation, in July, 1914, of a lysimeter field equipment. This permits of determination of both residual transformations and natural leachings.

The lysimeter tanks are illustrated in plate 2, figures 1 and 2. The tanks are placed in a single row along the top of the terrace, against which is built a supporting re-enforced concrete wall 7 feet high. This wall is paralleled by an outer concrete block wall of same length and height. At the upper end broad steps lead from the terrace above down to the concrete walk which runs parallel to and between the retaining and outer walls. The concrete walk has a trough running its entire length to care for the discarded surplus of leachings. At the other end a retaining wall extends the width of the walk at right angles thereto, connecting the two long walls. The enclosure is lighted by 7 windows placed in the long outer wall, by 6 panes of glass 26 by 32 inches in the roof, and by 2 sash doors, one in the upper end and the other in the long outer wall at its lower end. Electric wiring is also installed. The structure has a tight asphaltum-covered wooden roof. The leachings

from the treated soils are caught and stored in galvanized iron cans. These are painted with black asphaltum paint, and this in turn is coated with paraffin. Close-fitting tops prevent intrusion of light and evaporation of the leachings during their accumulation. The leachings are drained from the sand filters at the base of the tanks to the cans by means of block-tin tubes. As rainfall and leaching demand, the leachings are conveyed to the laboratory where their alkalinity and nitrate content are determined. Aliquots also are taken for acidified composite samples. These composites are then analyzed annually, or as often as may seem advisable.

That part of the installation devoted to the study of the activities of the several forms of lime embraces a total of 46 tanks, each tank being of 1/20,000 acre in area. Only 12 of the tanks are devoted to the present carbonation studies. One series contains surface soil only. This series, comprising 23 tanks, rests on a filter formed by 1 inch of clean sand. A second series of 23 tanks contain surface soil, underlaid by 12 inches of red clay subsoil, which in turn rests on the sand filters. The surface soil in each tank was about 8 inches deep when in loose tilth, and amounted to 100 pounds of moisture-free soil. As in the cylinder experiments, sufficient moist acid soil was dug nearby, screened through a $\frac{1}{4}$-inch mesh sieve and thoroughly mixed. While being carted to the lysimeter tanks, and also during the overnight period required for moisture determinations and calculations to a moisture-free basis, the soil was kept closely covered. The soil used in the tanks was taken from the same river bench from which the cylinder soil was obtained. It was, however, somewhat less cherty and more loamy than the soil of the cylinders.

In this experiment pulverized, freshly burnt lime was used against precipitated chalk as a check. In the cylinder experiments no appreciable difference was noted between the carbonation of the water-slaked lime applied and that of the burnt lime applied to and slaked in the moist soil. The parallel between CaO and $Ca(OH)_2$ was therefore not continued in the lysimeter experiments. Treatments were based upon applications of 8, 32 and 100 tons of CaO per 2,000,000 pounds of moisture-free soil. Analysis of the burnt lime gave an equivalence of approximately 93 per cent CaO and a trace of both MgO and CO_2. It is, of course, not to be contended that results obtained from the two larger treatments are capable of practical application. The use of the large amounts is, however, justified by academic and scientific requirements. With treatments of less than 8 tons, small and almost invariably plus analytical errors assume proportions relatively large in percentage, as compared with treatments given. But with the larger treatments and greater differences, we are more fully justified in drawing conclusions from analytical results. However, in the case of the heavier treatments greater homogeneity of the treated soil is essential and most likely the probability of error in the sampling of the smaller charges is coincidentally enhanced. Furthermore, the heavy treatments bring about conditions

markedly different from those resulting from the light applications. This is particularly true with reference to volume and differences of physical and biological nature.

As in the case of the cylinders, chemical equivalence was determined by titration and the treatments were sealed and conveyed to the field equipment in large bottles. In this experiment the entire 8 inches of moist soil and treatment were mixed in metal-lined boxes before placing in the lysimeters. At periods of 10, 17, 32, 49, 74, 218 days and at the end of 1, 2, 3 and 4 years subsequent to placing, samples were collected from the tanks which received

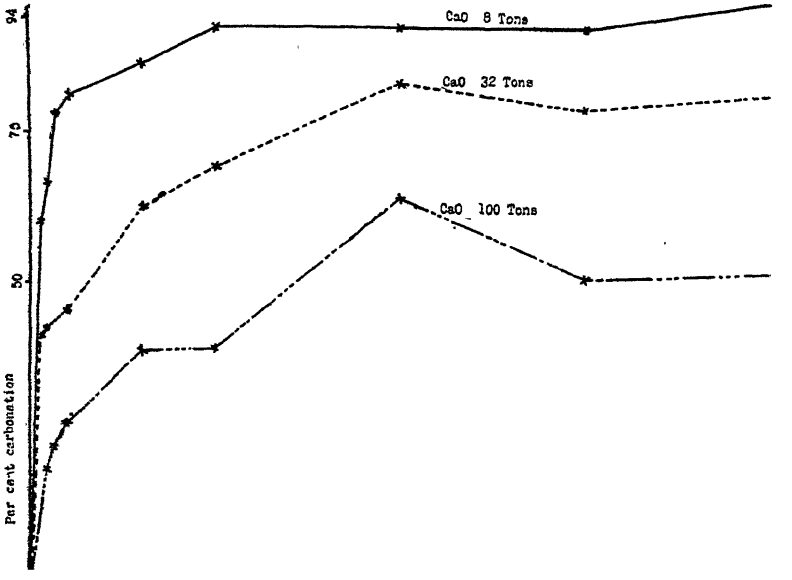

FIG. 3. SPEED OF THE REVERSION OF CaO TO $CaCO_3$, WHEN APPLIED TO THE CUMBERLAND LOAM IN THE LYSIMETERS, DISREGARDING THE LOSS BY LEACHING AND THE VARIATIONS IN WEIGHT OF THE APPLICATIONS (TABLES 25 TO 30)

oxide and carbonate check treatments. Borings were made with butter samplers to the full depth of the surface soil. The small samples thus secured were quickly dried by exposure in thin layers to the direct rays of the sun. They were then passed through a 0.5mm. sieve and analyzed for $CaCO_3$. At the end of 1, 2, 3 and 4-year periods, samples of the upper and lower halves of the entire depth were collected in order to study surface and subsurface carbonation. In the case of the heaviest applications, the applied materials resulted in a depth greater than the original 8 inches. The averages of duplicate determinations made upon the annual samplings from the upper and lower zones were used to obtain the average composition of the whole

depth. The residual carbonates resulting from applied CaO are compared periodically with the residual $CaCO_3$ of the precipitated carbonate checks in tables 25 to 30, inclusive. In these tables, the loss by leaching is not taken into consideration. It was, of course, almost impossible to correlate the

TABLE 25

Carbonation of freshly burnt pulverized unslacked lime when mixed with moist acid Cumberland loam soil without subsoil in field lysimeter tanks, during a period of 4 years as derived from determinations of residual carbonates, disregarding differential leachings; rate of applications 8 tons of 93 per cent CaO *per acre 2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3344	1	10 days				
3346	3	10 days				
3388	1	17 days	0.2151	0.4888	9,776	54.6
3390	3	17 days	0.3958	0.8955	17,910	
3430	1	32 days	0.2536	0.5763	11,526	63.2
3432	3	32 days	0.4001	0.9115	18,230	
3506	1	49 days	0.2929	0.6657	13,314	79.7
3508	3	49 days	0.3674	0.8350	16,700	
3631	1	74 days	0.3191	0.7252	14,504	77.6
3633	3	74 days	0.4111	0.9343	18,686	
4120	1	218 days	0.3314	0.7532	15,064	84.8
4122	3	218 days	0.3906	0.8877	17,754	
4162	1	1 year	0.3425	0.7783	15,568	92.8
4166	3	1 year	0.3691	0.8388	16,776	
4596	1	2 years	0.3068	0.6974	13,948	92.9
4598	3	2 years	0.3304	0.7508	15,016	
4894	1	3 years	0.282	0.641	12,820	93.2
4898	3	3 years	0.303	0.688	13,760	
5389	1	4 years	0.131	0.303	6,060	89.6
5393	3	4 years	0.149	0.338	6,760	

fractional leachings of the initial annual period with periodic determinations of residual carbonates during the relatively short periods. However, at the end of each annual period, complete analyses were made of the composite of leachings for the preceding year. These analyses afford the data of table 31.

Carbonation effected in the 8, 32 and 100-ton treatments of the lysimeter tanks

The treatments of CaO in the leaching tanks range from a minimum of 8 tons to a maximum of 100 tons per 2,000,000 pounds of soil. The effects

TABLE 26

Carbonation of freshly burnt, pulverized, unslacked lime when mixed with moist acid Cumberland loam soil overlying 12 inches of acid red clay subsoil in field lysimeter tanks, during a period of 4 years as derived from determinations of residual carbonate, disregarding differential leachings; rate of application 8 tons of 93 per cent CaO *per acre 2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3365	22	10 days	0.2417	0.5493	10,986	62.1
3367	24	10 days	0.3895	0.8852	17,704	
3409	22	17 days	0.2557	0.5811	11,622	65.1
3411	24	17 days	0.3929	0.8929	17,858	
3442	22	32 days	0.2691	0.6116	12,232	68.7
3444	24	32 days	0.3945	0.8966	17,932	
3518	22	49 days	0.2897	0.6584	13,168	74.9
3520	24	49 days	0.3863	0.8779	17,558	
3643	22	74 days	0.3535	0.8034	16,068	83.7
3645	24	74 days	0.4223	0.9597	19,194	
4132	22	218 days	0.3523	0.8007	16,014	86.4
4134	24	218 days	0.3830	0.8704	17,408	
4186	22	1 year	0.3339	0.7588	15,176	90.9
4190	24	1 year	0.3670	0.8341	16,682	
4620	22	2 years	0.2969	0.6804	13,608	91.1
4622	24	2 years	0.3279	0.7471	14,942	
4918	22	3 years	0.266	0.604	12,080	88.4
4922	24	3 years	0.300	0.683	13,660	
5413	22	4 years	0.283	0.643	12,860	101.1
5417	24	4 years	0.280	0.636	12,720	

produced are very striking. The heavier treatments produce conditions which are not common to the lighter treatments.

The 8-ton treatments will be first considered. The data obtained from the tanks receiving applications at this rate are recorded in tables 25 and 26.

During the first 10 days after treatment the production of carbonate amounted to over 68 per cent of that indicated by the check as being complete recarbonation. The speed of $CaCO_3$ formation in the tanks was decidedly less rapid than that observed where the same amount of lime was applied to the

TABLE 27

Carbonation of freshly burnt, pulverized, unslacked lime when mixed with moist acid Cumberland loam soil without subsoil in field lysimeter tanks, during a period of 4 years as derived from determinations of residual carbonates, disregarding differential leachings; rate of application 32 tons of 93 per cent CaO *per acre 2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3351	8	10 days				
3353	10	10 days				
3395	8	17 days	0.8128	1.847	36,940	38.4
3397	10	17 days	2.112	4.800	96,000	
3434	8	32 days	0.8259	1.877	37,540	38.7
3436	10	32 days	2.132	4.845	96,900	
3510	8	49 days	0.8119	1.843	36,860	38.8
3512	10	49 days	2.084	4.743	94,860	
3635	8	74 days	0.9851	2.2389	44,778	44.0
3637	10	74 days	2.2396	5.0886	101,772	
4124	8	218 days	1.2917	2.9357	58,714	61.1
4126	10	218 days	2.1137	4.8039	96,078	
4170	8	1 year	1.5201	3.4548	69,096	72.1
4174	10	1 year	2.1098	4.7950	95,900	
4606	8	2 years	1.6396	3.7264	74,528	80.8
4608	10	2 years	2.0285	4.6101	92,202	
4902	8	3 years	1.462	3.322	66,440	73.6
4906	10	3 years	1.987	4.516	90,320	
5397	8	4 years	1.465	3.329	66,580	72.4
5401	10	4 years	2.032	4.598	91,960	

cylinders in the same month of the preceding year. There was, however, a decided difference in the tilth maintained in the two experiments. As previously noted, only light showers fell upon the cylinder soils prior to complete carbonation of applied lime. As a result, the cylinder soils were of granular

structure during the period between application and the attainment of maximum carbonation. But the tank soils were saturated by heavy rains shortly after they were placed in the tanks and from the beginning they were, there-

TABLE 28

Carbonation of freshly burnt pulverized, unslacked lime when mixed with moist acid Cumberland loam soil overlying 12 inches of red clay acid subsoil in field lysimeter tanks, during a period of 4 years, as derived from determinations of residual carbonates, disregarding differential leachings; rate of application 32 tons 93 per cent CaO *per acre 2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3372	29	10 days				
3374	31	10 days				
3416	29	17 days	0.8673	1.971	39,420	43.2
3418	31	17 days	2.005	4.557	91,140	
3446	29	32 days	0.9378	2.131	42,620	45.2
3448	31	32 days	2.073	4.711	94,220	
3522	29	49 days				
3524	31	49 days				
3647	29	74 days	0.9818	2.2927	45,854	47.3
3649	31	74 days	2.1332	4.8482	96,964	
4136	29	218 days	1.3348	3.0336	60,672	63.3
4138	31	218 days	2.1410	4.8659	97,318	
4194	29	1 year	1.3760	3.1272	62,544	66.6
4198	31	1 year	2.0616	4.6859	93,718	
4628	29	2 years	1.729	3.9269	78,538	83.7
4632	31	2 years	2.0652	4.6936	93,872	
4926	29	3 years	1.598	3.631	72,620	80.9
4940	31	3 years	1.975	4.489	89,780	
5421	29	4 years	1.740	3.953	79,060	86.8
5425	31	4 years	2.005	4.556	91,120	

fore, in a much more compact condition. This difference in tilth and variation in pore space evidently had a marked influence upon oxidation and diffusion of soil atmosphere. Using the residual carbonates as the basis of comparison, only 92 per cent and 91 per cent carbonation was effected in

tanks 1 and 22, respectively, at the end of one year. If at the end of the first year, we add to the residual carbonate found in the oxide tank 1, the $CaCO_3$ equivalence of the excess of its total lime leached above that of its carbonate-check tank, a percentage carbonation of 95 is obtained. More than twice as

TABLE 29

Carbonation of freshly burnt pulverized unslacked lime when mixed with moist acid Cumberland loam soil without subsoil in field lysimeter tanks during a period of 4 years as derived from determinations of residual carbonates, disregarding differential leachings; rate of application 100 tons of 93 per cent CaO *per acre 2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3358	15	10 days				
3360	17	10 days	6.2978	14.313	286,160	
3402	15	17 days				
3404	17	17 days	6.2631	14.234	284,680	
3438	15	32 days	1.2339	2.804	56,080	19.7
3440	17	32 days	6.2499	14.204	284,080	
3514	15	49 days	1.4232	3.235	64,700	22.5
3516	17	49 days	6.2238	14.145	282,900	
3639	15	74 days	1.6789	3.815	76,300	26.6
3641	17	74 days	6.3160	14.354	287,080	
4128	15	218 days	2.4420	5.550	111,000	39.8
4130	17	218 days	6.1546	13.988	279,760	
4178	15	1 year	2.5059	5.695	113,900	39.8
4182	17	1 year	6.2946	14.305	286,100	
4612	15	2 years	3.9587	8.9971	179,942	63.1
4616	17	2 years	6.2715	14.2534	285,068	
4910	15	3 years	2.767	6.289	125,780	44.7
4914	17	3 years	6.185	14.057	281,140	
5405	15	4 years	3.677	8.357	167,140	60.1
5409	17	4 years	6.122	13.913	278,260	

much $CaCO_3$ was formed during the first month of contact as was formed during the succeeding 11 months. At the close of the succeeding annual periods, practically no further carbonation had ensued. It is difficult to explain the marked decrease in the residual carbonate of the surface soil of

the 8-ton oxide tank and its carbonate check after the third year. Though very marked, the decrease is consistent in the two tanks having no subsoil; but no such corresponding decrease took place in the case of the corresponding

TABLE 30

Carbonation of freshly burnt pulverized unslacked lime when mixed with moist acid Cumberland loam soil, overlying 12 inches of acid red clay subsoil in field lysimeter tanks, during a period of 4 years, as derived from determinations of residual carbonates, disregarding differential leachings; rate of application 100 tons of 93 per cent CaO *per acre·2,000,000 pounds of moisture-free soil*

LABORATORY NUMBER	TANK NUMBER	PERIOD BETWEEN TREATMENT AND SAMPLING	CO_2 IN MOISTURE-FREE SOIL	$CaCO_3$		CARBONATION AS COMPARED WITH $CaCO_3$ CHECK
				In moisture-free soil	Per acre 2,000,000 pounds of moisture-free soil	
			per cent	*per cent*	*lbs.*	*per cent*
3379	36	10 days				
3381	38	10 days	6.1770			
3423	36	17 days	2.2238			
3425	38	17 days	6.1337			
3450	36	32 days	1.0206	2.318	46,360	16.3
3452	38	32 days	5.9906	13.613	272,260	
3526	36	49 days				
3528	38	49 days	6.2523			
3651	36	74 days	1.6231	3.689	73,780	25.6
3653	38	74 days	6.2320	14.391	287,820	
4140	36	218 days	2.2965	5.219	104,380	37.1
4142	38	218 days	6.1964	14.077	281,540	
4202	36	1 year	2.6143	5.941	108,820	38.5
4206	38	1 year	6.2178	14.131	282,620	
4636	36	2 years	4.5476	10.3344	206,688	73.7*
4640	38	2 years	6.1688	14.0199	280,398	
4934	36	3 years	3.014	6.851	137,020	50.2
4938	38	3 years	6.003	13.643	272,860	
5429	36	4 years	2.626	5.968	119,360	43.0
5433	38	4 years	6.102	13.868	277,360	

* Probable error due to second sampling in hole made by previous sampling.

tank which contained subsoil. The year was decidedly subnormal, with reference to rainfall, and the leachings contained less than half as much lime as was contained in either that of the second or third year and only a fourth

of that of the first year. The tanks without subsoil were, as a rule, much drier than the corresponding ones with subsoil during the dry year and it is possible that greater absorption was thereby induced, as a result of a decreased tendency toward the effecting of hydrolysis and reversal of the reaction involved in the absorption of lime by the soil. To the eye, the water-holding capacity of the soils receiving 8-ton treatments appeared to have been materially increased. The treatment did not, however, develop flocculation and granulation to an extent sufficient to offset the tendency of the heavy precipitation to cause saturation, rather than the quick attainment of the optimum condition.

Considering next the data obtained from the 32-ton tanks as given in tables 27 and 28, we find a distinctly greater amount of absolute carbonation, though a considerably less percentage conversion to $CaCO_3$, than was shown by the analysis of the soils receiving 8-ton treatments. The percentage conversion of CaO to $CaCO_3$ increased very slowly after an initial rapid carbonation. During the first 17 days, an average of 40.8 per cent was obtained while a percentage of about 70, was reached at the end of the first year. An additional average increase of about 12 per cent occurred during the second year of contact after which no conclusive evidence of further carbonation was adduced. At the end of 4 years the leachings from the 32-ton tanks, without subsoil, were strongly impregnated with calcium hydroxide. The soils in these tanks underwent a wonderful physical transformation. Drainage from heavy rainfall is now usually complete within a few hours and shortly thereafter the soils attain a very dry state. As will be shown later, marked depression in the evidence of biological activities responsible for CO_2 production also resulted from the heavy treatments applied to these tanks. As a result, the large lime treatment is now contained in a soil often approaching almost air-dry conditions and of a distinct paucity of atmospheric CO_2. Because of this fact, together with the protective action of the dry soil in delimiting infusion of atmosphere, it is not remarkable that only about 80 per cent carbonation has been effected during the 2-year period of exposure.

We come next to consideration of the 100-ton treatments and to discussion of the data of tables 29 and 30. Here we find, though somewhat magnified, the same conditions which characterized the 32-ton applications. After 32 days of contact, more than twice as much actual carbonation is recorded, though a less percentage of conversion has been attained. The increase of carbonate effected during the second, third and fourth years of exposure has been less in percentage, but in extent far greater than that attained in the case of the 32-ton tanks. At the end of the fourth year of exposure, almost 50 per cent of the 100-ton applications is still uncarbonated. As was noted in the case of the 32-ton tanks, the leachings from the 100-ton surface-soil tanks were still strongly impregnated with calcium hydroxide at the end of the fourth year. The same restrictive tendencies noted in the case of the 32-ton tanks also obtain for the 100-ton treatments.

Leaching and other factors involved in comparisons of calcium carbonate residual from equivalent treatments of CaO *and* $CaCO_3$ *in the lysimeter experiments*

In table 31 are given the total amounts of lime leached from the lysimeters during each of the 4 annual periods, beginning July 15, 1914, together with a summary of losses incurred during the entire period of 4 years. These results should be considered in the light of their bearing upon the determination of residual carbonates.

The lysimeter experiments embody comparisons between excessive amounts of CaO in several forms of varying solubilities, over a long period of time, and hence introduce factors which are not involved in the cylinder and other studies. An untreated soil may be compared with one receiving and maintaining 8000 to 32,000 pounds of $CaCO_3$, the latter being the maximum of the cylinder applications and the minimum of the lysimeter treatments, without consideration being given to the weight of the applied material, when interpreting analytical results into terms of pounds per 2,000,000 pounds of soil. Even assuming that the above weights of applied carbonate were not decreased by disintegration through absorption of CaO and liberation of 44 per cent of the respective amounts, applications of 8000 to 32,000 pounds of $CaCO_3$ would amount to but 0.4 per cent and 1.6 per cent, respectively, on the original basis of 2,000,000 pounds of soil, as against 0.3984 per cent and 1.5748 per cent, respectively, on the basis of the augmented weights 2,008,000 and 2,032,000 pounds of soil. These respective differences of 0.0016 per cent and 0.0252 per cent may be considered as well within experimental error incident to sampling and analysis. In using a constant amount of soil and chemically equivalent but different weights of CaO and $CaCO_3$ at the rates of 32 and 100 tons of CaO, there were initially considerable variations in the actual moisture-free weights of the tank contents. These initial differences in moisture-free weights were in part decreased immediately by the hydration of the oxide, which occurred as a result of its access to the soil moisture, and they were still further decreased as the carbonate reaction proceeded. All of the calculations derived from the carbonate analyses were reported in pounds per acre, by the application of analytical results to the original acre, on the 2,000,000-pounds basis. Although close and well within error of sampling and analysis, in the case of the 8-ton treatments, the results are but roughly approximate in the case of the 32 and 100-ton treatments. In the lysimeter experiments there are involved a number of differential and intervening factors, some of which may be determined by chemical analysis, while others must needs be determined by process of elimination. In addition to the problem of variation of weight, the several factors involved may be considered under the following headings.

Absorption and interchange of bases. The absorption of lime may be considered as direct and also as substitutive. In the former case the calcium may be taken up by acid silicates and to some extent by silicic acid, as has

been pointed out by the writer (48), without interchange or liberation of other basic elements. In the latter case, the substitution of calcium for equivalent amounts of the alkali or alkali-earth elements may take place. Such substitution may or may not result in the loss of the liberated bases. It follows that unless enough lime is added to assure an excess of lime after taking care of the tendency of acid silicates to combine directly with lime, there will be little if any liberation of those bases locked up in native silicates. In so far as could be ascertained by examination of leachings, the substitutive activities of the several forms and amounts of lime have been established. These results will later be published. They show relatively little leaching of potassium and sodium. In the cylinder studies, and in the case of the 8-ton treatments in the lysimeters, it was determined that the actual variation in the amounts of direct absorption induced by equivalent amounts of CaO and $Ca(OH)_2$, as compared with $CaCO_3$, was not more than approximately 4 to 5 per cent. It may be, however, that the greater active mass of the solutions resulting from the 32 and 100-ton treatments of CaO has resulted in a more extensive direct absorption of calcium than that which occurred in the respective $CaCO_3$ equivalent checks, but this cannot be definitely established until final equilibrium between $Ca(OH)_2$ and CO_2 shall have been attained.

Sulfofication. In a preliminary report by the writer and associates (51), it was shown that there occurred a wide disparity between the amounts of sulfates leached from the tanks which received equivalent amounts of CaO and $CaCO_3$. It might thus be assumed that more engendered sulfuric acid was neutralized by the heavy carbonate treatments than by those of the corresponding caustic applications. However, since the solubility of $CaSO_4$ in water solutions of $Ca(OH)_2$ is decidedly less than in water solutions of $CaCO_3$, it does not necessarily follow that the sulfofication of native organic sulfur induced by the carbonate was greater than that induced by the hydrate. Certain unpublished data obtained by the writer have demonstrated that when sulfate salts are applied with excessive amounts of CaO, the amount of sulfur leached as sulfate is very small. This point may be considered more fully when complete carbonation shall have taken place.

Nitrification. The leachings of nitrates also have been determined periodically. The amounts of lime utilized in neutralizing the nitric acid generated represent appreciable and differential aggregates. In the final balancing of leachings and residual lime, these variations also will be considered and reported as a special phase of the study.

Solubilities of $Ca(OH)_2$ *and* $CaCO_3$. The total amount of lime leached from each tank is determined annually by analysis of a composite of the several separate leachings. In addition, the hydrate and carbonate contents are determined periodically by double titration of the separate leachings. The 32 and 100-ton burnt lime tanks continue to yield leachings strongly impregnated with $Ca(OH)_2$ after 4 years of exposure and these leachings carry away a great deal more lime than do those obtained from the carbonate

checks. Since an abundance of hydrated lime remains in the 32 and 100-ton CaO tanks, it cannot be assumed that the leached hydrate would have undergone carbonation, had it not been removed by leaching. Therefore, the several $Ca(OH)_2$ leachings cannot be calculated to $CaCO_3$ and added to the residual $CaCO_3$ in the soil of the respective tanks. Table 31 shows, in the case of the surface soil tanks, losses of 3603, 3566 and 3574 pounds per acre of total lime, calculated as $CaCO_3$, from the 8, 32 and 100-ton $CaCO_3$ tanks,

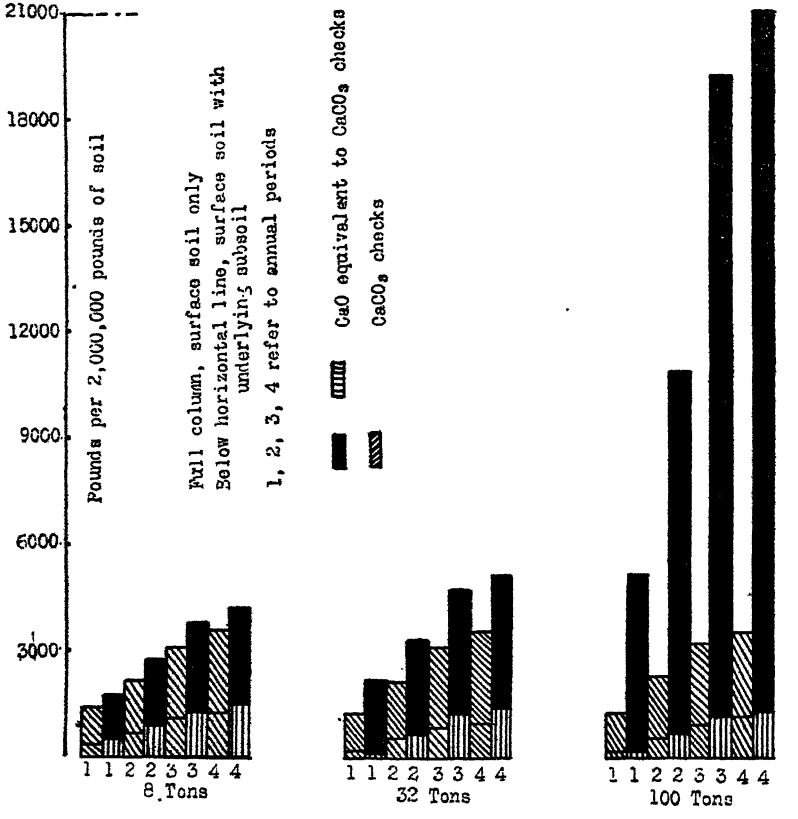

FIG. 4. TOTAL LIME SALTS, AS $CaCO_3$ EQUIVALENCE, LEACHED FROM LYSIMETERS DURING THE INITIAL 4 YEARS OF THE EXPERIMENT (TABLE 31)

respectively. These figures show a remarkable concordance. They demonstrate that with a sufficient surface offered by applied carbonate, the amount of application does not determine the extent of loss. This apparently is controlled rather by the concentration of the carbonated water content of the soil and its period of contact with the carbonate present. When these leachings are compared to the losses from the chemically equivalent CaO tanks without subsoil, we find a considerable variation between the outgo

TABLE 31

Amounts of calcium salts, calculated as $CaCO_3$, in leachings from oxide treatments, as compared to leachings from chemically equivalent carbonate checks during the first 4 years subsequent to applications

TANK NUMBER	TREATMENT		SUBSOIL	TOTAL VOLUME-WEIGHT OF ANNUAL LEACHINGS				CALCIUM-CARBONATE-EQUIVALENCES OF LIME SALTS LEACHED															
								1914–1915			1915–1916			1916–1917			1917–1918			Total for four years 1914–1918		
				1914 to 1915	1915 to 1916	1916 to 1917	1917 to 1918	Per liter	Per tank	Per 2,000,000 pounds of soil	Per liter	Per tank	Per 2,000,000 pounds of soil	Per liter	Per tank	Per 2,000,000 pounds of soil	Per liter	Per tank	Per 2,000,000 pounds of soil	Per liter	Per tank	Per 2,000,000 pounds of soil
	tons			*kgm.*	*kgm.*	*kgm.*	*kgm.*	*gm.*	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*	*gm.*	*gm.*	*lbs.*
1	CaO	8	None	69.7	136.8	170.1	81.5	0.5750	40.078	1767	0.1760	24.077	1062	0.1345	22.878	1009	0.1241	10.114	446	1.0096	97.147	4,284
3	$CaCO_3$	8	None	74.4	116.7	165.6	86.5	0.4285	31.880	1406	0.1615	18.847	831	0.1250	20.700	913	0.1187	10.268	453	0.8337	81.695	3,603
8	CaO	32	None	79.8	113.9	167.0	71.9	0.6161	49.165	2167	0.2500	28.475	1255	0.1840	30.728	1354	0.1509	10.850	478	1.2010	119.218	5,254
10	$CaCO_3$	32	None	81.5	118.5	181.6	76.8	0.3643	29.690	1309	0.1570	18.605	820	0.1275	23.154	1020	0.1232	9.462	417	0.7720	80.911	3,566
15	CaO	100	None	79.2	107.4	150.7	45.4	1.4821	117.382	5176	1.2090	129.847	5725	1.2350	186.115	8206	0.8196	37.210	1641	4.7457	470.559	20,748
17	$CaCO_3$	100	None	68.5	104.1	163.7	66.5	0.4232	28.989	1278	0.2175	22.642	998	0.1265	20.708	913	0.1313	8.731	385	0.8985	81.070	3,574
22	CaO	8	1 ft. clay	73.2	121.8	173.7	89.5	0.1553	11.368	501	0.0760	9.257	408	0.0535	9.293	410	0.0500	4.475	197	0.3348	34.393	1,516
24	$CaCO_3$	8	1 ft. clay	76.6	120.4	180.7	82.0	0.1035	7.928	350	0.0660	7.946	350	0.0545	9.848	434	0.0482	3.952	174	0.2722	29.674	1,308
29	CaO	32	1 ft. clay	73.2	108.9	157.3	75.9	0.0411	3.009	133	0.1185	12.905	569	0.0795	12.505	551	0.0536	4.068	179	0.2927	32.487	1,432
31	$CaCO_3$	32	1 ft. clay	72.3	110.4	173.1	85.3	0.0714	5.162	228	0.0730	8.059	355	0.0410	7.097	313	0.0393	3.355	148	0.2247	23.673	1,044
36	CaO	100	1 ft. clay	87.5	121.5	163.1	82.4	0.0411	3.596	159	0.0945	11.482	506	0.0640	10.438	460	0.0553	4.557	201	0.2549	30.073	1,326
38	$CaCO_3$	100	1 ft. clay	54.7	104.3	165.4	80.2	0.0714	3.906	172	0.0840	8.761	386	0.0475	7.857	346	0.0670	5.373	237	0.2699	25.897	1,141
45	Blank		1 ft. clay			165.5	69.2							0.0195	3.227	142	0.0134	0.927	*41	*0.0329	*4.154	*183
46	Blank		None			192.4	109.6							0.0330	6.349	280	0.0232	2.543	*112	*0.0562	*8.892	*392

* For 2 years only.

from equivalent applications of CaO and $CaCO_3$, at the rates of 32 and 100 tons. The 8-ton treatments of CaO gave a total loss of 4284 pounds of lime, calculated as $CaCO_3$ for the 4 years, as against 3603 pounds for its carbonate-equivalent check. The 32-ton CaO treatments lost 5254 pounds of $CaCO_3$ as against 3566 pounds for its carbonate-equivalent check; while the 100-ton CaO tank underwent an aggregate lime loss equivalent to 20,748 pounds of $CaCO_3$ as against 3574 pounds for its carbonate check.

Influence of subsoil. The foregoing losses were obtained by direct leachings from the surface soil without any estoppage by underlying subsoil. Were the correction of leached lime to be applied to residual carbonates, the amounts lost from the soil underlaid by subsoil would have to be assumed to be equivalent to those lost where there was no subsoil. The underlying clay subsoil has estopped a large amount of the lime which has passed from the surface soil and it cannot be assumed that the subsoil is saturated with reference to its tendency to absorb lime, until the leachings from the surface soil alone and those underlaid by subsoil are closely approximate in concentration. The total loss of lime, calculated as $CaCO_3$, during the 4-year period is 4284 pounds per acre for the surface soil alone as compared to 1516 pounds for the corresponding treatment of CaO at the rate of 8 tons per acre, where no subsoil was present; while the corresponding $CaCO_3$ checks gave $CaCO_3$-equivalent losses of 3603 and 1308 pounds, respectively. From the 32-ton CaO treatments, the losses of lime in terms of $CaCO_3$ per acre were 5254 pounds for the surface soil alone, as against 1432 pounds for the surface soil plus subsoil tank, while the corresponding carbonate treatments gave 3566 pounds and 1044 pounds, respectively, for surface soil only and soil underlaid by subsoil. Again, the surface soil treated with CaO at the rate of 100 tons per acre gave a $CaCO_3$-equivalent leaching of 20,748 pounds as against but 1326 pounds where the subsoil intervened; while the corresponding $CaCO_3$ checks gave only 3574 pounds for the shallow tank and but 1141 pounds for the deep tank containing both soil and subsoil.

It is thus apparent, that in an effort to effect a final balance when all hydrate shall have disappeared, the summation of data may be so utilized as to leave for determination by process of elimination, only the factor of absorption. As before mentioned the persistency of the hydrate has resulted in a constant contact between a strong $Ca(OH)_2$ solution, as contrasted with a dilute solution of $CaCO_3$ in weakly carbonated water, and it may be anticipated that the amounts of lime absorbed from the oxide admixtures will be shown to be considerably in excess of those absorbed from the carbonate treatments. From the determined residual carbonate content of the soil in each shallow tank, when all hydrate shall have disappeared, together with the known amounts and forms of lime leached, it will be possible by direct application of analyses to arrive at definite conclusions with reference to the different effects of $Ca(OH)_2$ and $CaCO_3$ upon the extent of absorption of CaO by the surface soil. However, in interpreting the data obtained from the subsoil

tanks, it will be necessary to assume that the leachings from the surface soil are equivalent to those from the respective identical treatments, where no subsoil was present to take up any of the lime which has passed through the surface soil. If this assumption is permissible, the extent of absorption effected by the subsoil may also be determined by difference, at a later period.

Until it shall be possible to make complete calculations, the pounds per acre data for the 32 and 100-ton treatments, as given in tables 27 to 30, inclusive, may be considered as being approximate with a tendency to appear higher than the real.

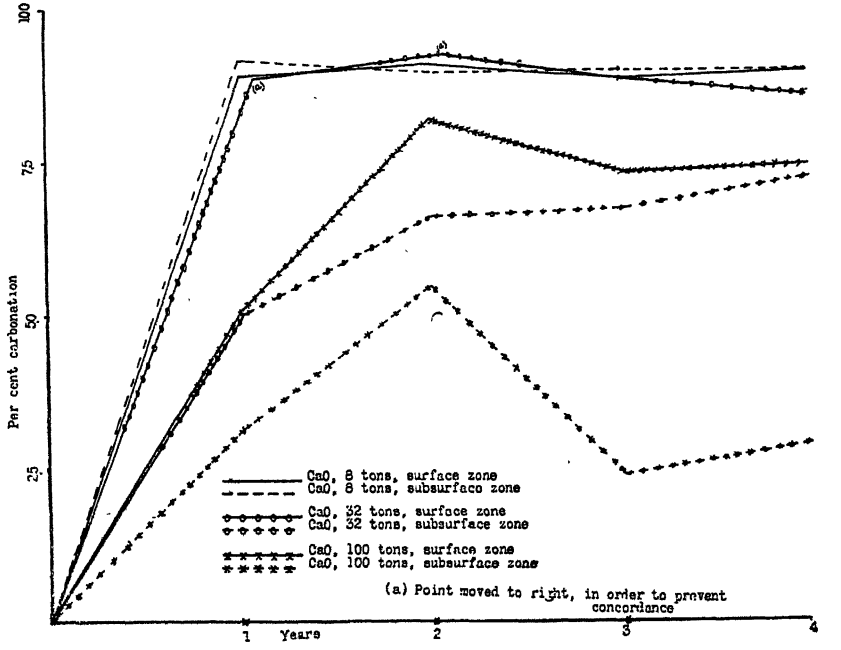

FIG. 5. CARBONATION OF CaO TREATMENTS IN SOIL SURFACES AND SUBSURFACES DURING 4 YEARS' EXPOSURE IN LYSIMETERS (TABLES 32 TO 35)

Carbonation of CaO *treatments effected in soil surfaces and subsurfaces during 4 years' exposure in lysimeters*

At the end of 1, 2, 3, and 4-year periods after placing, samples were taken from the upper and lower portions of the heavily treated lime tanks. The $CaCO_3$ checks were sampled in the same manner. As previously stated, treatments were mixed uniformly throughout the whole of the soil when the soils were placed in the tanks. The analyses of the upper and lower strata at the end of the 1, 2, 3, and 4-year periods are given in tables 32 to 35, inclusive. These analyses show uniform carbonate occurrence throughout the full depth

TABLE 32

Comparisons between extent of carbonation in upper half and that in lower half of an acid Cumberland loam soil in field lysimeter tanks, as derived from determinations of residual carbonates, disregarding differential leachings, at the end of 1 year's exposure; checks received chemically equivalent treatments of $CaCO_3$

SOIL WITHOUT SUBSOIL							SOIL WITH 1 FOOT OF CLAY SUBSOIL						
Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation	Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation
				per cent	*per cent*	*per cent*					*per cent*	*per cent*	*per cent*
4162	1	CaO 8 tons	Upper	0.3345	0.7602	91.27	4186	22	CaO 8 tons	Upper	0.3276	0.7218	87.37
4163	1	CaO 8 tons	Lower	0.3504	0.7964	94.25	4187	22	CaO 8 tons	Lower	0.3407	0.7721	91.72
4166	3	$CaCO_3$ 8 tons	Upper	0.3664	0.8327		4190	24	$CaCO_3$ 8 tons	Upper	0.3635	0.8261	
4167	3	$CaCO_3$ 8 tons	Lower	0.3718	0.8450		4191	24	$CaCO_3$ 8 tons	Lower	0.3704	0.8418	
4170	8	CaO 32 tons	Upper	1.9805	4.5011	93.59	4194	29	CaO 32 tons	Upper	1.7853	4.0575	86.09
4171	8	CaO 32 tons	Lower	1.0596	2.4082	50.38	4195	29	CaO 32 tons	Lower	0.9666	2.2877	49.13
4174	10	$CaCO_3$ 32 tons	Upper	2.1161	4.8095		4198	31	$CaCO_3$ 32 tons	Upper	2.0741	4.7139	
4175	10	$CaCO_3$ 32 tons	Lower	2.1033	4.7802		4199	31	$CaCO_3$ 32 tons	Lower	2.0490	4.6568	
4178	15	CaO 100 tons	Upper	3.1991	7.2707	50.44	4202	36	CaO 100 tons	Upper	3.2628	7.1882	50.92
4179	15	CaO 100 tons	Lower	1.8126	4.1195	29.03	4203	36	CaO 100 tons	Lower	1.9658	4.6950	33.12
4182	17	$CaCO_3$ 100 tons	Upper	6.3423	14.4143		4206	38	$CaCO_3$ 100 tons	Upper	6.2118	14.1177	
4183	17	$CaCO_3$ 100 tons	Lower	6.2468	14.1973		4207	38	$CaCO_3$ 100 tons	Lower	6.2238	14.1450	

TABLE 33

Comparisons between extent of carbonation in upper half and that in lower half of an acid Cumberland loam soil in field lysimeter tanks, without underlying subsoil, as derived from determinations of residual carbonates, disregarding differential leachings, at the end of 2 years' exposure; checks received chemically equivalent treatment of $CaCO_3$

SOIL WITHOUT SUBSOIL							SOIL WITH 1 FOOT OF CLAY SUBSOIL						
Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation	Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation
				per cent	*per cent*	*per cent*					*per cent*	*per cent*	*per cent*
4596	1	CaO 8 tons	Upper	0.2936	0.6673	94.7	4620	22	CaO 8 tons	Upper	0.2833	0.6552	91.4
4597	1	CaO 8 tons	Lower	0.3200	0.7274	91.3	4621	22	CaO 8 tons	Lower	0.3104	0.7055	91.2
4600	3	$CaCO_3$ 8 tons	Upper	0.3101	0.7048		4624	24	$CaCO_3$ 8 tons	Upper	0.3153	0.7166	
4601	3	$CaCO_3$ 8 tons	Lower	0.3506	0.7968		4625	24	$CaCO_3$ 8 tons	Lower	0.3404	0.7736	
4604	8	CaO 32 tons	Upper	1.9398	4.4086	98.5	4628	29	CaO 32 tons	Upper	1.8731	4.2570	90.3
4605	8	CaO 32 tons	Lower	1.3394	3.0441	65.5	4629	29	CaO 32 tons	Lower	1.5826	3.5968	77.0
4608	10	$CaCO_3$ 32 tons	Upper	2.0139	4.5770		4632	31	$CaCO_3$ 32 tons	Upper	2.0739	4.7136	
4609	10	$CaCO_3$ 32 tons	Lower	2.0430	4.6432		4633	31	$CaCO_3$ 32 tons	Lower	2.0564	4.6736	
4612	15	CaO 100 tons	Upper	5.0658	11.5132	80.7	4636	36	CaO 100 tons	Upper	5.2273	11.8780	83.3
4613	15	CaO 100 tons	Lower	2.8516	6.4809	45.5	4637	36	CaO 100 tons	Lower	3.8679	8.7907	63.8
4616	17	$CaCO_3$ 100 tons	Upper	6.2765	14.2648		4640	38	$CaCO_3$ 100 tons	Upper	6.2747	14.2607	
4617	17	$CaCO_3$ 100 tons	Lower	6.2665	14.2420		4641	38	$CaCO_3$ 100 tons	Lower	6.0628	13.7791	

TABLE 34

Comparisons between extent of carbonation in upper half and that in lower half of an acid Cumberland loam soil in field lysimeter tanks as derived from determinations of residual carbonates, disregarding differential leachings, at the end of 3 years' exposure; checks contained chemically equivalent treatments of $CaCO_3$

SOIL WITHOUT SUBSOIL							SOIL WITH 1 FOOT OF CLAY SUBSOIL						
Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation	Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation
				per cent	*per cent*	*per cent*					*per cent*	*per cent*	*per cent*
4894	1	CaO 8 tons	Upper	0.247	0.562	88.2	4918	22	CaO 8 tons	Upper	0.247	0.562	91.8
4895	1	CaO 8 tons	Lower	0.316	0.719	97.3	4919	22	CaO 8 tons	Lower	0.284	0.646	85.8
4898	3	$CaCO_3$ 8 tons	Upper	0.280	0.637		4922	24	$CaCO_3$ 8 tons	Upper	0.269	0.612	
4899	3	$CaCO_3$ 8 tons	Lower	0.325	0.739		4923	24	$CaCO_3$ 8 tons	Lower	0.331	0.753	
4902	8	CaO 32 tons	Upper	1.682	3.823	89.5	4926	29	CaO 32 tons	Upper	1.635	3.716	86.8
4903	8	CaO 32 tons	Lower	1.241	2.821	59.3	4927	29	CaO 32 tons	Lower	1.560	3.546	75.5
4906	10	$CaCO_3$ 32 tons	Upper	1.878	4.269		4930	31	$CaCO_3$ 32 tons	Upper	1.884	4.282	
4907	10	$CaCO_3$ 32 tons	Lower	2.095	4.762		4931	31	$CaCO_3$ 32 tons	Lower	2.066	4.696	
4910	15	CaO 100 tons	Upper	4.149	9.430	69.7	4934	36	CaO 100 tons	Upper	4.477	10.176	76.4
4911	15	CaO 100 tons	Lower	1.385	3.148	21.6	4935	36	CaO 100 tons	Lower	1.551	3.525	25.2
4914	17	$CaCO_3$ 100 tons	Upper	5.953	13.530		4938	38	$CaCO_3$ 100 tons	Upper	5.859	13.316	
4915	17	$CaCO_3$ 100 tons	Lower	6.417	14.584		4939	38	$CaCO_3$ 100 tons	Lower	6.147	13.970	

TABLE 35

Comparisons between extent of carbonation in upper half and that in lower half of an acid Cumberland loam soil in field lysimeter tanks, as derived from determinations of residual carbonates, disregarding differential leachings, at the end of 4 years' exposure; checks contained chemically equivalent treatments of $CaCO_3$

SOIL WITHOUT SUBSOIL							SOIL WITH 1 FOOT OF CLAY SUBSOIL						
Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation	Laboratory number	Tank number	Treatment	Section analyzed	CO_2 in soil	$CaCO_3$ in soil	Carbonation
				per cent	*per cent*	*per cent*					*per cent*	*per cent*	*per cent*
5389	1	CaO 8 tons	Upper	0.116	0.264	84.9	5413	22	CaO 8 tons	Upper	0.266	0.605	98.2
5390	1	CaO 8 tons	Lower	0.146	0.332	91.2	5414	22	CaO 8 tons	Lower	0.299	0.680	103.8*
5393	3	$CaCO_3$ 8 tons	Upper	0.137	0.311		5417	24	$CaCO_3$ 8 tons	Upper	0.271	0.616	
5394	3	$CaCO_3$ 8 tons	Lower	0.160	0.364		5418	24	$CaCO_3$ 8 tons	Lower	0.288	0.655	
5397	8	CaO 32 tons	Upper	1.664	3.782	83.9	5421	29	CaO 32 tons	Upper	1.746	3.968	89.3
5398	8	CaO 32 tons	Lower	1.265	2.875	61.3	5422	29	CaO 32 tons	Lower	1.733	3.938	84.4
5401	10	$CaCO_3$ 32 tons	Upper	1.983	4.507		5425	31	$CaCO_3$ 32 tons	Upper	1.955	4.443	
5402	10	$CaCO_3$ 32 tons	Lower	2.063	4.689		5426	31	$CaCO_3$ 32 tons	Lower	2.054	4.668	
5405	15	CaO 100 tons	Upper	5.318	12.086	85.9	5429	36	CaO 100 tons	Upper	3.800	8.636	62.4
5406	15	CaO 100 tons	Lower	2.036	4.627	33.7	5430	36	CaO 100 tons	Lower	1.452	3.300	23.8
5409	17	$CaCO_3$ 100 tons	Upper	6.194	14.076		5433	38	$CaCO_3$ 100 tons	Upper	6.094	13.850	
5410	17	$CaCO_3$ 100 tons	Lower	6.050	13.750		5434	38	$CaCO_3$ 100 tons	Lower	6.110	13.886	

* Not included in graph average.

of the soil which received CaO at the rate of 8 tons of CaO per 2,000,000 pounds of soil. The analyses do not, however, necessarily establish the fact that carbonation of the 8-ton treatments was effected with the same rapidity in the two strata. While the extent of carbonation was practically the same in the two strata after 1 year of exposure, it is quite possible that the carbonation effected within that interval may have been effected much more rapidly in one stratum than in the other.

The results obtained from the 32-ton tanks show a decided difference in the amounts of carbonate produced in the surface 4 inches, as compared to the amounts found in the lower 4-inch stratum. At the end of the first year about nine-tenths of the CaO of the upper 4 inches had been converted to $CaCO_3$ while about one-half of the lime in the lower stratum had been carbonated. At the end of the second year about 95 per cent of the upper stratum had undergone carbonation while the carbonation effected in the lower stratum amounted to about 70 per cent.

The results obtained from the 100-ton treatments are in accord with those secured from the 32-ton applications, in that much more extensive carbonation took place in the upper stratum than in the lower. About one-half of the lime present in the upper layer had undergone reversion to carbonate at the end of one year's exposure, while about four-fifths had become carbonated at the end of the second year. In the case of the lower stratum, about 30 per cent of the caustic form had been neutralized at the end of the first year of exposure, while at the end of the second year about one-half of the applied lime still remained in the caustic form. The distinctly greater carbonation effected in the upper stratum of each of the 32 and 100-ton tanks indicated that the CO_2 of the soil atmosphere of these tanks suffered reduction to a minimum. Hence, relatively greater infusion of atmosphere into the upper layer was responsible for the more extensive carbonation there effected. Moreover, the upper stratum would extract the CO_2 contained in rainfall, and perhaps dew as well. In other words, apparently the excessive caustic treatments effectuated conditions of partial sterilization, thereby necessitating dependence primarily upon infusion of atmosphere and precipitation as a source of CO_2 for the carbonation of CaO. This phase of the problem was then considered.

Influence of heavy treatments of CaO *upon the occurrence of atmospheric* CO_2 *in tank soils*

It seemed possible that, in the soils relatively dry as a result of the marked physical effect of the excessive lime treatments, causticity might persist because of protective action of $CaCO_3$ in spite of an abundance of atmospheric CO_2 in the soil. Again, the possibility of partial sterilization above mentioned seemed germane. Therefore, the atmosphere of the 12 tanks was analyzed at various periods during the 4 years. The samples were drawn off

from the bottom by means of the block-tin drainage outlets. Five hundred cubic centimeters were first drawn off and discarded in order to insure absence of error introduced by diffusion of atmosphere into the block-tin tubes. At first 500-cc. samples were then drawn off by means of a mercury siphon, dried and absorbed in $Ba(OH)_2$. Later the volumes of samples were increased to 2 litres and then 3 litres. Corrections were then made for observed temperature and pressure and later for moisture vapor, when a water siphon was used in place of mercury. The results are recorded in table 36.

The analyses of these atmospheres show a decided tendency of caustic lime to effect both an initial and a continued depletion of atmospheric CO_2. It does not appear, however, that the excess of $CaCO_3$ has resulted in a diminution of the CO_2 of the soil atmosphere. The 8-ton treatment had become almost, if not completely, carbonated in less than one year and it would seem, therefore, that in the absence of caustic lime, and with a probable increase of available nutrients, recovery from any possible partial sterilization would have occurred subsequent to the initial year.

In a comprehensive biological study of five soils, Hutchinson and MacLennan (38) used bacteria counts to determine the sterilizing effect of lime. They used amounts of CaO varying from 0.1 per cent to 1.0 per cent and concluded that partial sterilization follows in the wake of applications in excess of 0.3 per cent. They note, however, a somewhat rapid return to normal numbers. The complete carbonation of the burnt lime and the return to normality were apparently somewhat coincident. As judged by atmospheric-CO_2 content, the consistently smaller amounts of CO_2 found in the 8-ton treatments point to the conclusion that the full restoration of biological activities had not ensued even after a period of two years. There appeared one additional possibility which was suggested by the results obtained by Russell and Appleyard (58). These investigators found a marked difference between the CO_2 content of the soil atmosphere and the CO_2 absorbed by the surface moisture film. Russell and Appleyard observed an increase in pressure, upon standing, in the atmosphere above a moist soil which had been subjected to thorough evacuation. This was found to be due to the retarded evolution of a film-absorbed gas which proved to be almost pure CO_2. The absorption apparently was a surface phenomenon.

Treatment with caustic lime in amounts equivalent to 8 tons effected a marked flocculation of colloidal material which would naturally decrease the effective surface. It is, therefore, possible that bacterial activities may have returned to normal and that the lesser CO_2 content was due to physical alteration of the soil structure, and a resultant disturbance of the initial equilibrium between CO_2 content of atmosphere, free water and film water. The results are not surprising in the case of 32- and 100-ton treatments. The continued presence of so much uncarbonated lime would be expected to eliminate atmospheric carbonic acid gas and maintain a condition of partial sterility with minimum evolution of CO_2.

TABLE 36

Atmospheric CO_2 content of CaO and $CaCO_3$-treated loam soil of tanks at periods between 1914 and 1918; results calculated to per cent of CO_2 by weight, dry air, at 0° C. and 760 mm. pressure

DATE OF SAMPLING OF ATMOSPHERE	SOIL WITHOUT SUBSOIL							SOIL WITH 1 FOOT OF CLAY SUBSOIL						
	Treatment							Treatment						
	CaO 8 tons	$CaCO_3$ = CaO 8 tons	CaO 32 tons	$CaCO_3$ = CaO 32 tons	CaO 100 tons	$CaCO_3$ = CaO 100 tons	Blank	CaO 8 tons	$CaCO_3$ = CaO 8 tons	CaO 32 tons	$CaCO_3$ = CaO 32 tons	CaO 100 tons	$CaCO_3$ = CaO 100 tons	Blank
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
June 30, 1916	0.262	0.328	0.046	0.452	0.045	0.405		0.438	0.576	0.094	0.448	0.096	0.629	
July 14, 1916	0.269	0.309	0.045	0.219	0.045	0.223		0.332	0.386	0.092	0.266	0.088	0.350	
October 12, 1916	0.147	0.169	0.043	0.191	0.000	0.170		0.126	0.169	0.043	0.168	0.022	0.166	
April 18, 1917	0.179	0.179	0.000	0.271	0.047	0.217	6.147	0.271	0.503	0.139	0.813	0.093	0.604	0.767
May 18, 1917	0.000	0.022	0.000	0.023	0.000	0.160	0.159	0.137	0.207	0.046	0.160	0 046	0.023	0.545
September 19, 1917	0.245	0.667	0.134	0.313	0.091	0.407	0.837	0.293	0.159	0.249	0.204	0.113	0.453	0.272
May 15, 1918	0.134	0.173	0.038	0.211	0.026	0.313		0.070		0.237	0.147		0.684	
August 31, 1918	0.133	0.146	0.013	0.139	0.000	0.186	0.238	0.152	0.300	0.118	0.179	0.113	0.252	0.173
Average	0.171	0.249	0.040	0.227	0.032	0.260	1.845	0.227	0.329	0.127	0.298	0.082	0.395	0.439

An additional point worthy of notation is suggested by the data of table 36. These data indicate that the atmosphere of the tanks underlaid by subsoil was considerably richer in CO_2 than was that of the soils without underlying subsoil. The atmosphere was sampled in both cases by aspiration from the bottom by means of the drainage outlets, and only small initial withdrawals were required to effect a constant composition. This constant was maintained after drawing quantities equivalent to a large multiple of the volume used for analysis. In the case of the subsoil tanks it follows, then, that the atmosphere of the dense clay of the subsoil was drawn off rather than the atmosphere of the surface soil. From this it appears that the atmosphere of the dense clay subsoil, almost free of organic matter, is richer in CO_2 than is the atmosphere of the surface soil. This suggests two possibilities. Selective absorption, or rather surface condensation, of CO_2 may be evidenced. Again, the lower temperature and the probable increased pressure characteristic of the subsoil may result in a more highly carbonated soil water. Reduction of pressure, incident to aspiration, would tend to effect release of CO_2 thus held in solution. Furthermore, the heavily lime-treated surface soil yields a leaching strongly impregnated with bicarbonate of lime, and this salt is still being absorbed by the subsoil, with a liberation of CO_2.

TUMBLER EXPERIMENTS TO DETERMINE RAPIDITY OF THE CARBONATION OF HYDRATED LIME EXPOSED ON THE SURFACE OF SOIL, AS COMPARED TO THAT EFFECTED UPON HYDRATED LIME INCORPORATED WITH THE SOIL

The data obtained from the foregoing lysimeter studies show that carbonation of burnt lime was more extensive in the upper half than in the lower half of the surface soil, when large amounts were simultaneously and uniformly mixed throughout the entire 8 inches of Cumberland loam. The nature of the experiments, and the equipment required, together with the character of certain other involved comparisons necessitated the thorough mixing of soil and treatments at the beginning of the experiments. This was true for both the leaching tanks and the naturally drained cylinders. These two series of experiments did not, therefore, permit of comparison between the rate of carbonation of burnt or hydrated lime applied to and left upon the surface, and that obtained by incorporating the oxide or hydrate in the soil. This phase of the problem was then attacked under the following conditions of laboratory control. Glass tumblers $4\frac{1}{2}$ inches high were used as containers. Two soils, a Volusia silt loam and a Dunkirk clay loam, were used in this work. Both of these soils are of glacial till origin and both were obtained in the near vicinity of the Cornell Station Farm. Both soils were gray in color and acid to litmus paper.

It was, of course, necessary to take into consideration the fact that if acid soils were used, a comparison could not be drawn quantitatively between

the carbonation of a definite amount of burnt lime applied and mixed throughout the soil and the carbonation resulting from an equal amount of burnt lime applied to and left on the soil surface. With an admixture of acid soil and lime there would occur absorption and fixation of lime which could not then be directly carbonated. With many soils the entire application of economical amounts of CaO thus applied, might be quickly absorbed from the hydrate by acid-reacting soil materials, leaving none to undergo carbonation.

In making dry admixtures, however, this would not hold true. Nor would there be appreciable absorption from undisturbed surface contact of lime with relatively small soil surface in the case of moistened but unleached acid soils. It was, therefore, necessary to eliminate or minimize the question of lime absorption by means of preliminary treatment of the acid soils to be utilized in the experiments. The plan adopted was as follows. Freshly sampled soils were brought to the laboratory, spread thinly, and permitted to air-dry. They were then sifted through a 2.5-mm. sieve and thoroughly mixed in order to insure uniformity of composition. The air-dry soils were next mixed with dry carbonated-water-treated precipitated $CaCO_3$ and then wetted to the consistency of soupy paste in galvanized iron containers. Two hundred and twenty-five pounds of Volusia soil and 612 gm. of $CaCO_3$ were mixed and moist contact continued with frequent stirrings for 18 days. Two hundred and thirty-four pounds of the Dunkirk soil and 637 gm. of $CaCO_3$ were treated, as was the Volusia silt loam. The Dunkirk clay was in moist contact with an excess of $CaCO_3$ for a period of 38 days. The amounts of $CaCO_3$ used represented field treatments equivalent to 12,000 pounds per acre 2,000,000 pounds of soil. Guided by lime-requirement determinations, it was empirically determined that this amount would insure neutralization of the immediate lime requirement, and also furnish a determinable excess of carbonate. At the end of the specified periods of contact the soils were poured out in thin layers on concrete floors and permitted to air-dry with frequent rakings. The alkaline air-dried soil was again passed through a 2.5-mm. sieve and thoroughly mixed. The flocculation effected by the $CaCO_3$ treatment produced a soil finely powdered as compared to the initial soil. The $Ca(OH)_2$ treatments were based upon *surface areas*, rather than upon the weight per acre of any definite depth. Applications at rates of *4 and 8 tons per acre surface* were then computed into grams per area of glass containers. A definite quantity of soil was used throughout giving a constant depth of $3\frac{1}{2}$ inches. In one series the lime was thoroughly mixed to this depth while in another series the lime was applied to the surface and evenly spread. At sampling periods the surface treatments were mixed with the soil. They were then sifted through a 1-mm. sieve, bottled and analyzed in a manner similar to that followed in the case of treatments where soil and lime were in intimate contact throughout.

The plan of treatment included consideration of the moisture factor, as compared to air-dry conditions; and the protective action of a sand mulch

to reduce aeration as compared with unrestricted exposure. An attempt was also made to insure permanent sterile conditions, during exposure, thus eliminating nitrification and generation of CO_2, and thus to differentiate between carbonation effected by atmospheric CO_2 and that produced biologically within the soil.

In considering the question of an antiseptic which would not involve absorption and various chemical transformations, *a priori* considerations resulted in the elimination of all the known effective materials except formaldehyde. Nor was this substance assumed to be ideal for the purpose intended. The anticipations and precautions attendent upon its use will be considered in the discussion of results. Twenty per cent formaldehyde, acid-free, was used.

In order that pore space might be as nearly constant and comparable as possible, in the dry conditions and in the two moist treatments, it was determined empirically to what volume the soil should be compacted so that expansive and contractive tendencies of wetting would be practically neutralized. This degree of compactness would probably be less conducive to oxidation and intra soil aeration and resultant carbonation of the hydrated lime included in the soil mass, than would be the case were the same soils freshly tilled in the field. It was necessary, however, to anticipate the compacting which would result from repeated wettings in order to effect comparable compactness in the three conditions maintained. Furthermore. there was necessarily less exposure to wind movement in the indoor exposure studies than would have been the case in the field. In studying the factors influencing carbonation it was, therefore, intended to effect conditions which would be comparably correct, rather than to secure results which, in actual rapidity of carbonation, would be in close harmony with field conditions.

The Volusia and Dunkirk soils were not run simultaneously. The former was exposed in a well ventilated greenhouse from September 1, 1915, to December 8, 1915. The latter was exposed in a large attic room from December 29, 1915, to March 22, 1916. The glass containers were embedded in an improvised soil of black well leached cinders. The artificial soil was leached to prevent any carbonate contamination by cinder dust or particles becoming incorporated in the treated soils. Each specified treatment was applied in quadruplicate and one of the quadruplicates was taken at each of the designated intervals. When sampled the entire content of each tumbler was turned out into agate-ware pans, immediately placed in electric ovens and dried overnight at 105°C. On removal from the oven, each tumbler's contents were immediately passed through a 1-mm. sieve and thoroughly mixed by rolling. Portions for analyses were then preserved in 8-ounce bottles, sealed with paraffin. The following scheme was carried out in the tumbler exposures:

1. Air-dried soil mixed throughout with $Ca(OH)_2$ at rate of 4 tons of CaO per acre of surface.
2. Air-dried soil and surface application of $Ca(OH)_2$ at the rate of 4 tons of CaO per acre of surface.
3. Same as 1 with an inch of CO_2-free quartz sand as mulch over lime.
4. Same as 2 with an inch of CO_2-free quartz sand as mulch over lime.
5. Air-dried soil mixed throughout with $Ca(OH)_2$ at the rate of 8 tons of CaO per acre of surface.
6. Air-dried soil and surface application of $Ca(OH)_2$ at the rate of 8 tons of CaO per acre of surface.
7. Same as 5 with an inch of CO_2-free quartz sand as mulch over lime.
8. Same as 6 with an inch of CO_2-free quartz sand as mulch over lime.

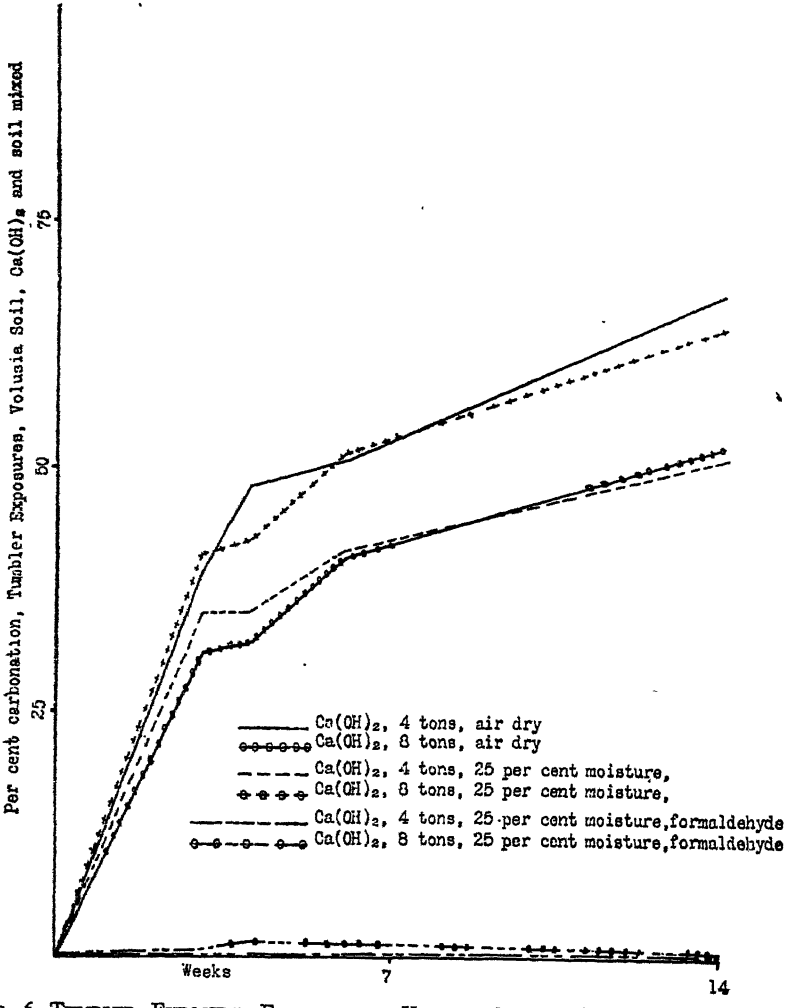

FIG. 6. TUMBLER EXPOSURE EXPERIMENTS, VOLUSIA SOIL—$Ca(OH)_2$ AND SOIL MIXED (TABLES 37, 38 AND 39)

TABLE 37

Speed of carbonation of 4 and 8 tons of CaO (*as* $Ca(OH)_2$) *per acre surface when mixed throughout 3½ inches of air-dry alkaline Volusia silt loam, as compared with carbonation of the same amount when applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
3	37.9	2769	81.6	5961	31.7	2316	49.3	3601	30.8	4500	85.1	12,433	19.2	2805	25.8	3769
4	47.8	3492	79.8	5829	33.3	2433	61.6	4500	31.7	4631	90.8	13,266	23.8	3477	34.2	4997
6	50.4	3682	82.7	6041	43.1	3148	83.4	6092	40.5	5817	90.9	13,280	29.4	4295	45.8	6591
14	67.3	4916	87.5	6012	56.1	4098	90.7	6625	52.0	7597	92.3	13,485	43.3	6326	62.5	9031

TABLE 38

Speed of carbonation of 4 and 8 tons of CaO (*as* $Ca(OH)_2$) *per acre surface when mixed throughout 3½ inches of alkaline Volusia silt loam maintained at 25 per cent moisture content, as compared with the carbonation of the same amount applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
3	41.1	3002	46.3	3382	31.9	2330	31.4	2294	35.1	5128	34.4	5026	23.5	3433	19.7	2788
4	42.3	3090	66.0	4821	40.1	2929	36.2	2644	35.1	5128	34.8	5084	28.6	4178	22.1	3229
8	51.1	3733	83.0	6063	53.4	3901	39.3	2871	41.3	6034	62.1	9073	28.9	4222	22.9	3346
14	63.7	4653	87.4	6385	61.6	4500	74.0	5406	50.4	7363	67.8	9906	41.2	5909	50.9	7436

TABLE 39

Speed of carbonation of 4 and 8 tons of CaO (*as* $Ca(OH)_2$) *per acre when mixed throughout 3½ inches of alkaline Volusia silt loam maintained at 25 per cent moisture content with 20 per cent formaldehyde, as compared with the carbonation of the same amounts when applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
3	0	0	15.2	1110	0	0	11.5	840	0.6	88	17.5	2558	1.1	161	9.3	1359
4	0	0	15.6	1140	0	0	18.4	1344	1.5	219	16.9	2479	0.9	131	10.0	1461
6	0	0	13.5	986	0	0	17.9	1308	1.1	161	17.6	2571	1.3	190	12.5	1826
14	0	0	16.8	1227	0	0	12.6	920	0.5	73	19.2	2805	0	0	18.0	2630

After application of the above treatments, four glasses of each treatment were left air-dry, four of each treatment were wetted to 25 per cent moisture content with freshly distilled water and the same number were brought to 25 per cent moisture content with 20 per cent formaldehyde. The glasses containing the wetted treatments were maintained at constant weight by additions of CO_2–free distilled water and 20 per cent formaldehyde, respectively. Plate 3 shows the Dunkirk soil series at the beginning of the experiment.

Carbonation of lime effected in tumbler exposures

The data derived from the tumbler exposure experiments are given in tables 37 to 44, inclusive.

The exposures with the Volusia soil will be first considered. Inspection of table 37 shows that the surface application of $Ca(OH)_2$ carbonated much more rapidly than did the hydrate which was mixed throughout the dry soil, in both 4 and 8-ton treatments. In the dry condition the biological activities would be exceedingly small, and the lime would necessarily draw from the air the CO_2 required for the reversion of the hydrate to the carbonate. Hence, it would be expected that the restricted diffusion of atmosphere into pore spaces would result in less carbonation than would be effected in the case of the surface applications. This is further emphasized by the lesser carbonation which transpired, where diffusion was restricted by appli-

cations of a 1-inch coarse sand mulch. In each case the greatest per cent of carbonation per unit of lime was effected during the initial 3-week interval. In the surface applications, devoid of mulch, about 80 per cent of theoretical carbonation resulted within the first 3 weeks. The reversion to carbonate

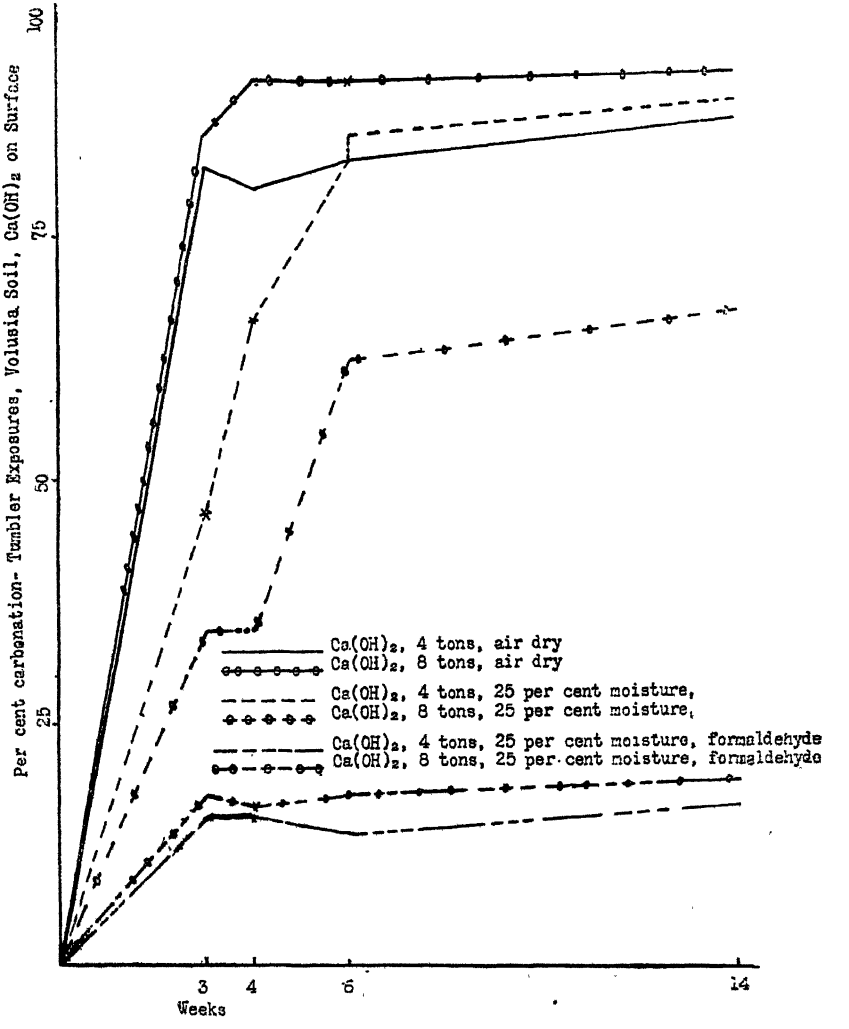

FIG. 7. TUMBLER EXPOSURE EXPERIMENTS, VOLUSIA SOIL—$Ca(OH)_2$ ON SOIL SURFACE (TABLES 37, 38 AND 39)

was slow beyond that time. In those tumblers in which lime was mixed throughout the soil, and also where the mulches were applied, carbonation was slower initially and less ultimately with a more gradual increase, than in the case of the surface applications. In the day treatments the carbo-

nation came to an apparent equilibrium without effecting theoretical carbonation, in the same manner as did the first exposures recorded in table 10.

With the addition of the water, in the tumblers where the moisture content was maintained at 25 per cent, additional factors are involved. The water in this set was added from the top, an air vent being afforded by a small glass tube extending to the bottom of the tumbler. In order to prevent the absorption of the entire amount of water by the upper portion of the soil, thus leaving a dry bottom layer which would pull from the moist strata, the initial wetting was beyond the condition maintained as a constant. This precaution resulted in a mass almost devoid of subsequent cracks. In these wet treatments the surface carbonation was also greater than that found within the soil. The compactness of the soil and the fact that pore space was diminished by the added water, would tend to restrict the infusion of atmosphere into these moist soils. Furthermore, the carbonation of the mixed 4-ton treatments was not materially altered by the presence of the mulch. It would, therefore, appear that the carbonation effected within the body of the soil receiving 4 tons is to be attributed in a large part to the biological evolution of carbon dioxide. On the other hand, however, there occurred the possibility of partial sterilization having been produced. For, while the 4 and 8-ton treatments are not excessive, as surface applications, they assume the proportions of 8 and 16 tons per acre, 7 inches, respectively, when mixed throughout $3\frac{1}{2}$ inches of soil. When we consider the 8-ton treatments, we find a difference. In this case the sand mulch has lessened the extent of reversion of $Ca(OH)_2$ to carbonate, indicating a sterilizing effect and a dependence upon atmospheric infusion as a source of CO_2.

We come now to the tumblers in which formaldehyde was used. In the treatments accorded this set, an endeavor was made to produce and maintain sterile soil conditions, at the same time, permitting free access to atmosphere. A careful preliminary survey of positive antiseptics was made. From such a search it seems impossible to secure a substance which would not introduce errors through either absorption or side reactions. Though difficulties were anticipated, it was decided to use acid-free formaldehyde, diluted 1 to 1. The possibility that the inherent oxidative power of the soils would cause oxidation of formaldehyde to formic acid was considered and determined qualitatively. For this purpose an extra set of treatments and $CaCO_3$ checks were enclosed in rubber-stoppered Erlenmeyer flasks, containing intake and outgo glass tubes to which were sealed glass stopcocks. After a period of some weeks the atmosphere of the sterile $CaCO_3$-treated check flasks was drawn off and found to be heavily impregnated with CO_2. This is further shown in the determination of residual carbonates of the checks after treatment with formaldehyde, as given in table 43. This demonstrated that the formic acid generated had attacked the precipitated carbonate of the preliminary treatment. It may be, however, that acid compounds were generated from contact of amino and amido compounds with formaldehyde. This phe-

nomenon was also demonstrated by analysis of $CaCO_3$-treated tumbler checks, for residual carbonate as shown in table 43.

Spoehr (62) noted that formaldehyde underwent oxidation with the production of formic acid on exposure to the atmosphere. He also noted the formation of a sugar in the presence of $Ca(OH)_2$, even in the dark. Such a condition existed in the mixed moist treatment and it is quite possible that acetic acid may have also been formed from the sugar produced by action of lime upon formaldehyde. Butlerow (9) is cited as having made the same observation. Sadtler and Coblentz (60) state that formaldehyde polymerizes into a white crystalline mass known as para-formaldehyde, and that Fisher observed the formation of an inactive sugar, which he designated as anacrose of the formulum $C_6H_{12}O_6$. Both of these observations were also made in the tumbler studies. Plate 4 shows the accumulation of para-formaldehyde upon the surface of the Volusia soil after 14 weeks. In addition, the capillary rise brought to the soil surface a yellowish syrupy substance, which formed caramel upon heating.

While the efficiency of the formaldehyde was proved by the determined sterility of the 8 tumblers exposed during the 14 weeks, the complications above cited nullified the apparent indications advanced by the determinations of CO_2, as given in table 39. Because of the lack of carbonate increase from the mixed 4-ton treatments and the minute amounts formed in the 8-ton treatments it appears that as fast as the transformation of formaldehyde to formic acid occurred, the free acid was neutralized by the hydrated lime. The surface carbonation shown indicates that the vapor pressure of the formaldehyde was not sufficiently strong to prevent carbonation from the atmosphere. The fluctuations which were carefully checked, may possibly be accounted for by capillary rise of the formic acid or other acids generated near the top of the soil or by the oxidation of the sugar above-mentioned.

Carbonation of $Ca(OH)_2$ *when applied upon surface and when mixed throughout Dunkirk clay loam*

The trend of the results of the exposure of the Dunkirk soil is very similar to that shown in the case of the Volusia. In the dry treatments the carbonation has been more rapid on the surface than in the body of the soil. The protective action of a sand mulch also is well exemplified. In the 4-ton and 8-ton surface applications, with moisture maintained at 25 per cent, the percentage carbonation amounted to 94.5 and 99.2, respectively. In these exposures the successive additions of water, added in maintaining a constant soil moisture supply, were responsible for repeated disruptures of the surface coatings of carbonate, thus permitting almost complete carbonation.

In the case of the moist treatments the same relationships likewise hold. Again, in the treatments to which formaldehyde was added, the acid produced from oxidation, accelerated by extensive surface, has prevented any

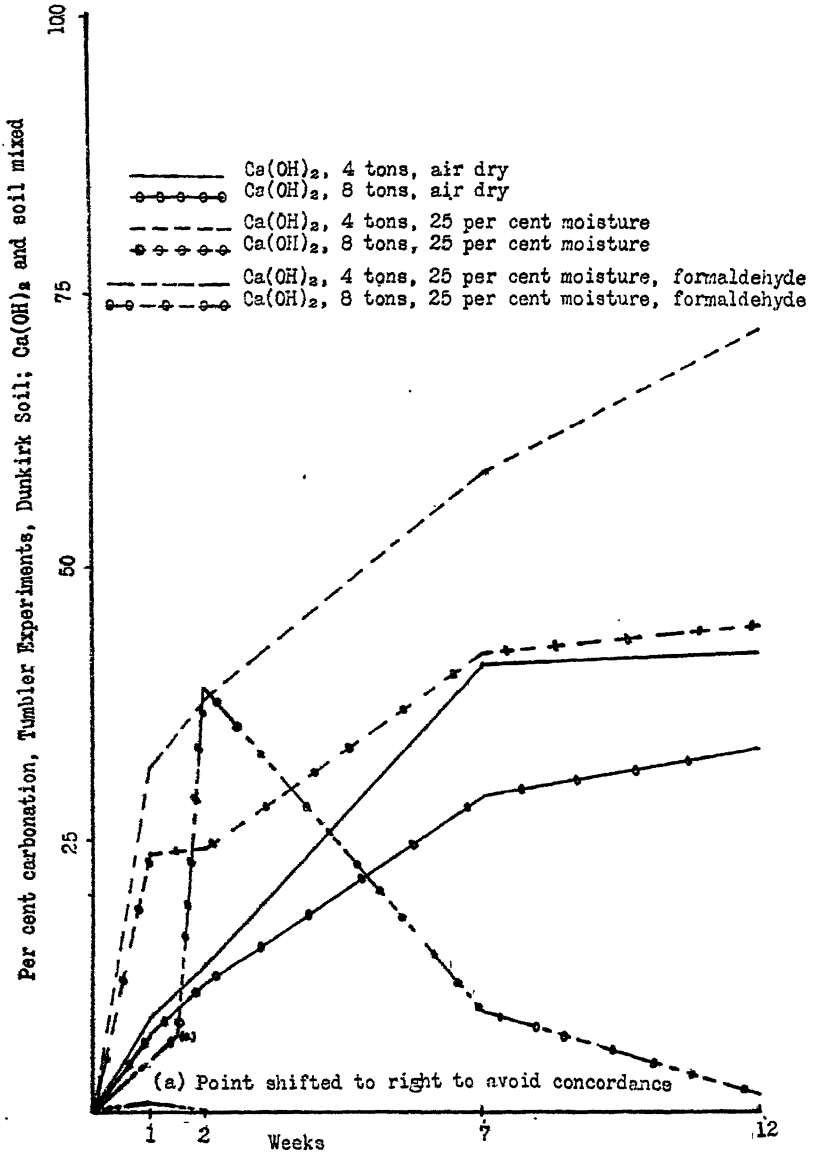

FIG. 8. TUMBLER EXPOSURE EXPERIMENTS, DUNKIRK SOIL—$Ca(OH)_2$ AND SOIL MIXED (TABLES 40, 41 AND 42)

TABLE 40

Speed of carbonation of 4 and 8 tons of CaO (*as* $Ca(OH)_2$) *per acre surface when mixed throughout 3½ inches of air-dry alkaline Dunkirk clay loam, as compared with carbonation of the same amounts applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
1	8.6	628	14.0	1023	9.1	665	9.3	679	7.1	1037	7.9	1154	5.2	760	6.9	1008
2	13.3	971	24.2	1768	11.0	804	13.1	957	11.6	1695	17.8	2601	7.5	1096	11.9	1739
7	40.6	2966	56.5	4127	32.5	2374	39.2	2864	28.5	4164	45.6	6662	22.5	3287	28.3	4135
12	41.9	3061	56.5	4127	37.3	2725	53.7	3923	32.8	4792	46.8	6837	29.5	4310	38.8	5669

TABLE 41

Speed of carbonation of 4 and 8 tons of CaO (*as* $Ca(OH)_2$) *per acre surface when mixed throughout 3½ inches of alkaline Dunkirk clay loam maintained at 25 per cent moisture content, as compared with the carbonation of the same amounts when applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
1	31.2	2289	64.0	4675	14.4	1052	20.9	1527	23.6	3448	36.1	6,299	20.7	3024	11.5	1,680
2	37.2	2717	83.9	6129	35.6	2601	32.5	2374	24.1	3521	61.8	9,029	20.9	3053	18.3	2,674
7	57.8	4222	85.2	6224	48.1	3514	80.8	5902	41.6	5978	94.6	13,821	27.8	4062	48,8	7,130
12	67.9	4960	94.5	6913	50.5	3689	90.0	6575	43.8	6399	99.2	14,493	36.5	5333	72.3	10,563

TABLE 42

Speed of carbonation of 4 and 8 tons of CaO (as $Ca(OH)_2$) *per acre surface when mixed throughout 3½ inches of alkaline Dunkirk clay loam maintained at 25 per cent moisture content with neutral 20 per cent formaldehyde, as compared with carbonation of the same amounts when applied to and left upon the surface*

PERIOD OF EXPOSURE	METHOD OF TREATMENT															
	CaO 4 tons mixed		CaO 4 tons surface		CaO 4 tons mixed and sand mulch		CaO 4 tons surface and sand mulch		CaO 8 tons mixed		CaO 8 tons surface		CaO 8 tons mixed and sand mulch		CaO 8 tons surface and sand mulch	
	Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated		Carbonated	
	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface	Per cent	Pounds per acre surface
weeks																
1	0	0	28.0	2045	0	0	8.0	584	7.1	1038	7.5	1096	4.8	701	7.3	1067
2	0.8	58	29.9	2183	0	0	12.2	891	38.2	5582	23.9	3492	4.9	716	9.2	1344
7	0	0	32.2	2352	0	0	28.0	2045	9.2	1344	44.2	6458	4.8	701	14.6	2133
12	0	0	19.0	1388	0	0	22.6	1651	1.6	234	40.5	5917	1.6	234	24.6	3594

consistent or extensive accumulation of carbonate. The foregoing experiments indicate that when burnt lime is left upon the soil surface *in the amounts and of fineness comparable to that used experimentally*, it will carbonate more quickly than when incorporated with or under a dry mulch. The same relationship would appear to hold also if the soils were compacted by rain.

Constancy of checks used in tumbler experiments

In comparing the production of carbonates from the applied hydrates, it was not assumed that the checks would remain constant for a long period under conditions of moist contact. It has been shown that some soils and certain soil constituents, alkaline, sterile, and free of organic matter will continue to evolve CO_2 when left for relatively long periods in contact with $CaCO_3$. For this reason separate checks were used in the work, one set for dry treatments, one for moist treatments and a third for the formaldehyde tumblers.

Some further absorption of calcium appeared to follow as a result of continued contact of the soil and an excess of the carbonate up to the 2 weeks interval in the case of the Dunkirk clay. The preliminary treatment of the Volusia silt loam seemed sufficiently intense, however, to satisfy its tendency to absorb lime within the time of experimental conditions.

The marked effect produced by the acids generated by oxidation of formaldehyde or sugar is very plainly demonstrated by the large decrease of $CaCO_3$ in the formaldehyde checks.

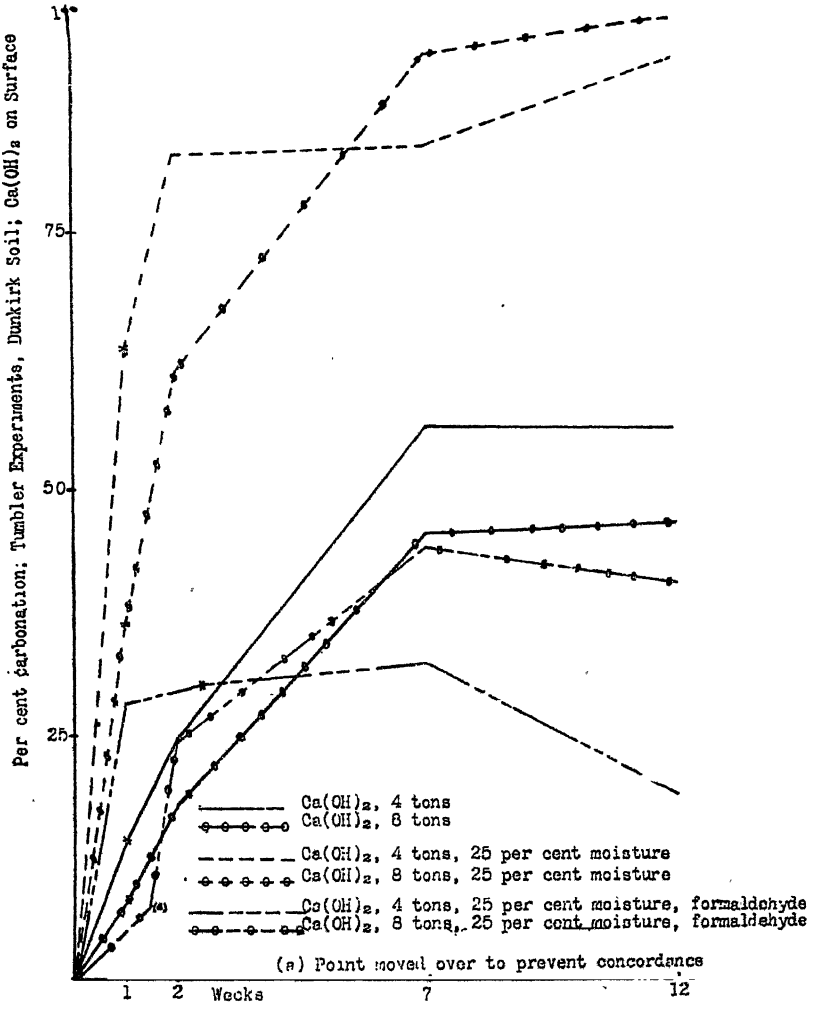

FIG. 9. TUMBLER EXPOSURE EXPERIMENTS, DUNKIRK SOIL—$Ca(OH)_2$ ON SOIL SURFACE (TABLES 40, 41 AND 42)

Supplementary tumbler experiments with calcareous Dunkirk soil; studies as to the source of the CO_2, *which combines with the* $Ca(OH)_2$ *lying upon the soil surface*

In the foregoing tumbler exposure experiments there was established no definite demarkation between the amount of CO_2 derived from atmospheric diffusion and that engendered biologically within the soil. Furthermore, in the maintenance of a definite moisture content, the surface applications of calcium hydrate were subjected to wetting. A supplementary set of Dun-

TABLE 43

Comparisons of $CaCO_3$ contents of alkaline air-dry soils, used in tumblers as the basis of treatments supplemented by 4 and 8-tons CaO equivalent check treatments of $CaCO_3$ and the same soils subsequent to further moist contact; 25 per cent moisture content being maintained by additions of distilled water and also 20 per cent formaldehyde

PERIOD OF CONTACT	AIR-DRY		MOISTURE 25 PER CENT DISTILLED H_2O		MOISTURE 25 PER CENT FORMALDEHYDE	
	4 tons	8 tons	4 tons	8 tons	4 tons	8 tons
Dunkirk clay loam*						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Initial	1.55	3.04				
2 weeks			1.40	2.80	1.21	2.69
12 weeks			1.40	2.82	0.81	2.19
Volusia silt loam						
Initial	1.65	3.21				
4 weeks			1.64	3.20	1.37	3.00
14 weeks			1.64	3.21	1.27	2.65

* Dunkirk blank initially 0.41; after two weeks 0.155; and after 12 weeks 0.025 per cent carbonate, with formaldehyde treatments.

TABLE 44

Carbonation in supplementary set of Dunkirk clay loam; $Ca(OH)_2$ exposed on surface to atmospheric diffusion and to soil-atmosphere CO_2 as compared to exposure to soil-atmosphere CO_2 alone; water and 20 per cent formaldehyde used for maintenance of moisture constant supplied by capillarity

PERIOD OF EXPOSURE	MOISTURE CONTENT 25 PER CENT MAINTAINED BY ADDITIONS OF CO_2-FREE DISTILLED H_2O				MOISTURE CONTENT 25 PER CENT MAINTAINED BY ADDITIONS OF 20 PER CENT FORMALDEHYDE			
	CaO 4 tons exposed	CaO 4 tons closed	CaO 8 tons exposed	CaO 8 tons closed	CaO 4 tons exposed	CaO 4 tons closed	CaO 8 tons exposed	CaO 8 tons closed
	Per cent carbonation	Per cent carbonation	Per cent carbonation	Per cent carbonation	Per cent carbonation	Per cent carbonation	Per cent carbonation	Per cent carbonation
weeks								
2	62.6	19.8	64.7	11.8	46.1	10.4	47.4	6.7
7	76.6	24.7	79.0	14.1	70.5	25.0	59.7	14.1

kirk soil was, therefore, introduced in order to differentiate in part between the two sources of CO_2 and also to eliminate the necessity of wetting the surface charges of lime.

In the bottoms of a number of tumbler containers $\frac{1}{2}$ inch of clean sand was placed. Upon this was placed a uniformly compacted $3\frac{1}{2}$-inch depth of soil. Through the center of the soil and to the bottom of the glass extended the beveled edge neck of a 9-cm. funnel. This funnel was used as a means of introducing water and formaldehyde into the sand at the bottom of the

tumblers and thus, by capillarity, to insure the maintenance of moisture content. Upon the surface of the soils in the tumblers was placed a layer of sand $\frac{1}{4}$ inch in thickness. Upon this were spread charges of $Ca(OH)_2$ at the rates of 4 and 8 tons per acre surface. In this way direct contact between treatment and moist soil was prevented. One series, comprising one-half of the tumblers, was left exposed. The remaining tumblers were completely covered with black paper which was then made air-tight by means of vaseline-paraffin mixture. The containers are shown in figure 10. Excepting the probable meagre amount of air which would diffuse down the small neck of the funnel and up into the soil, the charges would in one case be subjected to only the biologically evolved CO_2. On the other hand, the surface applications, devoid of the impervious paper covering, were probably more extensively bathed by air movements than were the paper-covered surface treat-

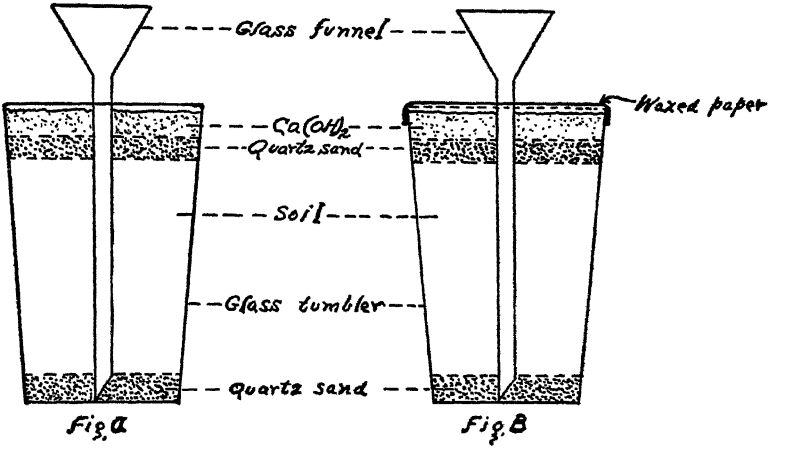

FIG. 10. EQUIPMENT USED IN COMPARING SURFACE CARBONATION WITH RESTRICTED AND UNRESTRICTED DIFFUSION

ments, because of greater opportunity afforded for diffusion between soil air and the atmosphere, and also because of the greater lateral movement of atmosphere across the surface.

The data from this exposure experiment are contained in table 44. These results show some diffusion of CO_2 from the alkaline moist soil to the overlying layer of lime, where no external exposure was permitted. The larger part of the gain in CO_2 comes, however, from the atmosphere. In the case of the formaldehyde tumblers, the carbonation effected was much greater than in the former cases. In this set the capillary rise of any acid produced was stopped by the layer of sand intervening between soil and $Ca(OH)_2$. The partial neutralization of acetic acid by $Ca(OH)_2$ was thus prevented. The tumblers were proved by culture plate tests to be sterile from the effects of formaldehyde. Therefore, no CO_2 could be evolved biologically. However, the action of

oxidized acids upon the initial $CaCO_3$ of the calcareous soil would furnish some CO_2 and effect carbonation of the adjacent surface hydrate. Additional CO_2 may have been derived through oxidative processes inherent to the soil and by a giving up of CO_2 condensed upon the surfaces of the soil particles.

The data indicate that hydrated lime lying upon the surface of a moist soil will, in reverting to carbonate, derive most of its absorbed CO_2 from the lateral movement of air across the surface, rather than from the outward diffusion of soil atmosphere.

This phase of the problem was further studied in the field under conditions simulating those of practice.

FURTHER STUDIES CONCERNING THE SOURCE OF THE CO_2 WHICH COMBINES WITH THE $Ca(OH)_2$ LYING UPON THE SOIL SURFACE

As previously stated, the foregoing data, obtained in the supplementary tumbler experiments, were secured with a soil which had been rendered alkaline by subjection to preliminary treatment with precipitated $CaCO_3$. This preliminary treatment and the method of control were such as to establish conditions which would be considerably at variance with those that would be encountered in the field. The problem was, therefore, attacked from another angle.

In applying lime in the field, it is quite probable that applications are most generally made upon either a dry dust or soil mulch secured by cultivation, or upon a natural compact soil mulch. In either case the lime applied would be in contact with a comparatively dry soil, toward which it would be relatively inactive. It is therefore probable that most of the finely divided hydrate applied to a soil surface would all be converted to the carbonate form before its incorporation with the soil, unless the working-in were done very shortly after the application. For, it has been shown that reasonable applications of lime are very quickly converted to the carbonate when mixed with the soil, and that surface applications are still more rapidly converted. Disregarding rainfall and dew, this surface-lying lime has but two sources from which it may obtain the CO_2 required for its carbonation, namely, diffused soil atmosphere and aerial movements across the soil surface.

With the above points in mind, surface applications of lime were exposed in the following manner. Mason jar covers were used as containers for the exposed charges of lime. A layer of glass wool was first laid upon the bottom of the container. Upon this was placed a definite amount of dry sifted sawdust with which had been mixed 0.3 gm. of $Ca(OH)_2$. One-half of the number of jar covers used were perforated before the introduction of the lime charges in the sawdust mixture: the others were unchanged. The layer of glass wool intervening between the lime and the surface of the moist soil was intended to simulate the inactive surface mulch to be expected under field

conditions. The perforated and unperforated containers were then simultaneously placed side by side over the surfaces of four soils. Protection against rainfall was afforded without restriction of lateral movement of air. After 7 days of exposure the jar-top containers were placed in desiccators and conveyed to the laboratory for carbonate determinations. The experiment was then repeated with four other soils during a subsequent period of the same number of days. The data secured from these exposures are given in table 45.

TABLE 45

Effect of diffusion of soil atmosphere from field cylinders, in accelerating the carbonation of surface applications of lime, where direct contact of lime and soil was prevented; exposure for 7 days, August, 1918

SOIL		TREATMENTS JUNE, 1913; 5 YEARS PRIOR TO PRESENT EXPERIMENT	CARBONATION		
Type	Condition		Perforated bottoms permitting upward diffusion of soil atmosphere	Unperforated bottoms preventing upward diffusion of soil atmosphere	Increase induced by movement of soil atmosphere through perforations
			per cent	*per cent*	*per cent*
Cookeville fine sandy loam.	Optimum	None	70.54	52.94	17.60
Crossville fine sandy loam...	Optimum	None	80.93	59.50	21.43
Gallatin brown loam.....	Optimum	None	75.82	62.70	13.12
Jackson sandy loam.....	Optimum	None	79.33	69.73	9.60
Cumberland loam.....	Moist	Manure, 48 tons	45.61	25.59	20.02
Cumberland loam.....	Moist	Manure, 48 tons; Limestone, 16 tons	52.34	32.31	20.03
Cumberland loam.....	Moist	Hydrated lime, 8 tons	46.26	23.54	22.72
Cumberland loam.....	Moist	Limestone, 16 tons	49.90	27.57	22.33
Average.....			62.59	44.23	18.36

Discussion of data of table 45

In the case of each of the eight soils, there occurred a greater carbonation where the perforations permitted an upward diffusion of the soil atmosphere as supplementary to that of aerial movements across the surface of the charges of $Ca(OH)_2$ lying upon the top of the soil. The carbonation effected in the eight charges having access to the diffused soil atmosphere resulted in an average of 62.59 per cent of the absolute, while the eight charges not subject to the diffused CO_2 gave an average of 44.23 per cent of the possible. This increase, attributable to the diffusion through the perforations, amounted to an average of 18.36 per cent of the theoretical carbonation, or 41.3 per cent of the carbonation effected in the unperforated-container exposures. These data are consistent and support those of table 44. Nevertheless, they do not con-

clusively demonstrate that the greater carbonation induced by proximity to the soil atmosphere is of necessity attributable to CO_2 diffused from the soil. It might be assumed that there occurred a diffusion of both CO_2 and moisture from the soil to the surface charges contained in the perforated tops. On the other hand, it might be assumed that only moisture vapor passed from the soil and to the surface charges of lime, through the perforations in the covers. For, under the latter conditions, the $Ca(OH)_2$ would have reverted to the carbonate more rapidly in the perforated covers than in the unperforated ones, where no supplementary moisture was available to accelerate conversion of the exposed hydrate from solid to solution phase. In other words, were the adjacent perforated and unperforated tops bathed by aerial atmosphere and by it alone, the more moist $Ca(OH)_2$ would effect a more rapid extraction of CO_2 from the air passing over the surfaces. This deduction follows from the preliminary laboratory studies in which it was shown that it is essential to have sufficient moisture to maintain the solution of the hydrate in case the carbonate reaction is to proceed without hindrance. In the field, under humid conditions and with minimum wind velocity, the moisture, chemically liberated in the hydrate-to-carbonate reaction, might remain sufficiently long to aid in the maintaining of the solution phase of the hydrate. However, under average conditions, the atmosphere, unsaturated with reference to moisture, would rapidly conduct the chemically liberated H_2O away from the surface of the liberating hydrate. Hence, it is obvious that acceleration of the carbonate reaction induced by the adjacency of the hydrate to the soil atmosphere might possibly be attributable to the activating influence of diffused moisture, even to the exclusion of CO_2 of soil atmosphere derivation.

In order to clarify this point, the procedure which yielded the data of table 45 was slightly modified and amplified, in such a way as to afford an abundance of CO_2-free moisture to surface charges of $Ca(OH)_2$, at the same time protecting these from direct diffusion of soil atmosphere. In addition to the exposures in perforated and unperforated containers, a third series of containers was simultaneously and adjacently exposed. This supplementary series was made from pairs of jar tops, two nested covers being used for each charge exposed. The inner and upper top was perforated, while the outer and lower one was unperforated. The latter contained a definite amount of asbestos, which was moistened with CO_2-free water. The space between the outer and inner containers was filled with melted parafin, so that no moisture could escape from the reservoir except through the perforations in the bottom of the inner and upper container, the latter containing $Ca(OH)_2$. The hydrate in this experiment was mixed with asbestos instead of dry sawdust as in the former experiment. Charges of 0.5 gm. were used instead of 0.3 gm. as formerly. Three conditions were thus maintained in this exposure experiment. Intimate mixtures of lime and asbestos were exposed at the soil surface: (a) without direct access to CO_2 of the soil atmosphere, or to

TABLE 46

Relative effects of moisture vapor and CO_2 of diffused soil atmosphere from field cylinders in accelerating the carbonation of surface applications of hydrated lime, where direct contact of lime and soil was prevented

SOIL	WEATHER CONDITIONS	SEASON OF EXPOSURE	NUMBER OF DAYS OF EXPOSURE	TREATMENT JUNE, 1913—5 YEARS PRIOR TO PRESENT EXPERIMENT	CARBONATION		
					Unperforated bottoms preventing upward diffusion of moisture vapor and CO_2	Perforated bottoms permitting upward movement of moisture from moist asbestos	Perforated bottoms permitting upward diffusion of both moisture vapor and CO_2 from soil
					per cent	*per cent*	*per cent*
Cumberland loam	Rainy	October	7	Manure, 48 tons	78.90	87.93	92.04
Cumberland loam	Rainy	October	7	None	82.45	84.70	86.96
Jackson sandy loam	Rainy	October	7	None	62.44	73.49	74.46
Cumberland loam	Fair	November	4	Manure, 48 tons	53.56	60.99	66.31
Cumberland loam	Fair	November	4	None	47.04	51.40	76.80
Cherokee sandy loam	Fair	November	4	None	53.02	58.50	79.48
Jackson sandy loam	Fair	November	4	None	55.44	58.66	75.12
Average					61.84	67.95	78.74

supplementary moisture, except that of the aerial atmosphere; (b) without direct access to CO_2 of the soil atmosphere, but with access to supplementary moisture as furnished by the asbestos reservoir; (c) with direct access to both CO_2 and moisture of the soil atmosphere. The exposures were sheltered without restriction of lateral air movement. The results are incorporated in table 46.

The data of table 46 show that, in the case of each of the seven soils used, the same relationship is maintained in the respective speeds of the carbonate reaction, as induced by the three conditions of exposure. The unperforated containers gave an average carbonation of 61.84 per cent of the possible; the containers with perforations permitting movement of moisture from the asbestos reservoir gave 67.95 per cent of that possible, as an average; while the containers with perforated bottoms permitting access of both CO_2 and moisture from the soil gave an average percentage of 78.74. It is thus evident that the soil-derived moisture was instrumental in accelerating the carbonate reaction. If it is assumed that as much moisture passed through the perforations above the asbestos as passed from the soil through similar perforations, or if the moisture diffused from the asbestos was sufficient to give maximum speed of carbonation with the atmospheric CO_2 available, then it follows that the hydrate derived from the soil atmosphere the lesser part of the CO_2 with which it combined. This conclusion is further fortified by the exposures which gave the data of table 47.

Supplementary exposures demonstrating that CO_2 of the soil atmosphere is in part responsible for the carbonation of $Ca(OH)_2$ lying on the surface of a soil mulch

In studying further the source of the CO_2 which effects the carbonation of soil surface applications of $Ca(OH)_2$ the perforated jar top nests of table 46 were again used. In one set of these nested containers the outer and lower receptacle contained a definite amount of CO_2-free asbestos which rested upon a thin bed of glass wool; while the upper and inner jar cover contained a definite amount of $Ca(OH)_2$ mixed with the asbestos, the mixture resting upon a thin layer of glass wool as in the case of the lower and outer jar of the nest. In another set a definite amount of $Ca(OH)_2$ was mixed with the asbestos of both the upper and lower jar covers. In this way the upward diffusion of the CO_2 of the soil atmosphere was unimpeded in one case, while in the other the lower mixture of $Ca(OH)_2$ and asbestos served as a trap protecting the upper charge of $Ca(OH)_2$. The exposures of the surface charges of lime with and without the underlying trap layers were made during mild winter days of December and January at the Tennessee Station farm. The results from the two series of exposures are given in table 47.

The data incorporated in this table show that the surface charges of $Ca(OH)_2$ which were underlaid by the $Ca(OH)_2$ filters, were less extensively

carbonated than were those underlaid with asbestos only. Thus the lower layer effected an estoppage of CO_2 diffusion from the soil atmosphere to the surface-lying charges of $Ca(OH)_2$. The asbestos-lime filter layer showed a considerable increase in its CO_2 content subsequent to exposure. In fact, the average increase in the filter layer represented an amount greater than the average differential between the protected and unprotected surface layers. This may be due in part to the two-fold surface exposure offered by the two

TABLE 47

Movement of CO_2 from the soil atmosphere to surface-lying charges of $Ca(OH)_2$ as indicated by the trapping of CO_2 by means of a $Ca(OH)_2$- asbestos-mixture filter intervening between the soil surface and an overlying exposed charge of $Ca(OH)_2$ mixed with asbestos; perforated-bottom metal containers; soil quite moist

SOIL	DATE OF EXPOSURE	$Ca(OH)_2$ CHARGE	CO_2 GAIN IN $Ca(OH)_2$ FILTER	CARBONATION	
				Surface charge with blank asbestos filter	Surface charge with $Ca(OH)_2$ asbestos filter
		gm.	*per cent*	*per cent*	*per cent*
Cumberland loam	December 19–23	0.6		79.44	78.04
Cherokee sandy loam	December 19–23	0.6		69.05	63.43
Jackson fine sandy loam	December 19–23	0.6		50.27	48.57
Cherokee sandy loam	January 30–February 3	2.0	10.95	69.04	67.16
Cumberland loam	January 30–February 3	2.0	11.87	52.25	50.39
Jackson fine sandy loam	January 30–February 3	2.0	10.64	53.27	52.38
Cumberland loam	January 30–February 3	2.0	16.00	53.40	51.06
Average			12.37	60.96	58.72

separate $Ca(OH)_2$ layers, the filter layer being 2 gm. in each case, and it might be assumed that not all of the surface downward movement of CO_2 was taken care of by the upper layer. Had the exposures been made during the summer, the actual amount diffused to the surface would doubtless have been more extensive.

Taken in connection with the preceding tables, the data of table 47 may be considered as proving definitely that a movement of soil atmosphere to surface-lying $Ca(OH)_2$ is only in part responsible for the conversion of the hydrate to the carbonate.

FURTHER STUDIES AS TO THE SPEED OF THE CARBONATE REACTION, IN THE CASE OF THE SURFACE EXPOSURES OF $Ca(OH)_2$, AS COMPARED TO THAT OBTAINED WHEN THE HYDRATE IS WITHIN THE SOIL MASS

The data obtained from the analyses of the upper and lower strata of the soil of the lysimeter tanks showed no appreciable difference in the occurrence of carbonates in the two strata, at the end of one year of exposure, where applications were made at the rate of 8 tons of CaO per 2,000,000 pounds of soil. Almost, if not, complete carbonation of the lime incorporated with the soil was effected in both strata. While these analyses demonstrated but little difference in the ultimate $CaCO_3$ content of the respective layers at the end of the initial annual period, they do not establish the relative speeds of carbonation in the two strata. However, the differential was determined in each instance of the tanks receiving CaO at the rates of 32 and 100 tons. These heavier treatments showed incomplete carbonation and a consistently greater $CaCO_3$ content in the upper stratum. Furthermore, in the tumbler-exposure experiments a consistently greater carbonation was effected in the surface applications, as compared with the carbonation effected from equal amounts mixed throughout the soil mass, under both dry and moist conditions.

Because of the greater atmospheric CO_2 content of a normal soil, as compared with that of the atmosphere above its surface, this would not be expected; unless it is assumed that the unrestricted movement of the aerial atmosphere would tend to offset the greater percentage CO_2 of the more restricted soil atmosphere. The continued occurrence of hydrated lime in the 32 and 100-ton treatments suggested several possibilities. It seemed possible that complete carbonation might be retarded by the protective action of $CaCO_3$ coatings around the numerous minute lime particles, even in the presence of an abundance of atmospheric CO_2. However, such a pseudo equilibrium would be frequently disrupted by rainfall as is evidenced by the continued leaching of hydrate from these heavily limed tanks and also by the almost complete carbonation attained in the wetted surface soils in the tumbler experiments. The moisture content of the tanks receiving the heavy lime treatments is usually low, because of quick evaporation after light rains and rapid drainage, followed by rapid evaporation, after heavy rains. This would tend to favor such a condition of restricted carbonation because of a diminished CO_2 generation. However, the atmospheric CO_2 determinations of table 36 demonstrated the poverty of CO_2 in the soil atmosphere of the heavily limed tanks. This marked decrease of atmospheric CO_2 strongly indicated a diminution of biological activities, to be attributed to the partial sterilization effected and maintained by the lime. There remained, however, the possibility that the less restricted movement of the surface atmosphere would bring to the surface applications greater amounts of CO_2 than would be diffused by the soil atmosphere to the lime incorporated within the soil. In order to elucidate this point it seemed advisable to submit hydrated lime to the surface

and soil atmospheres, without permitting actual contact between lime and soil. This was done in two ways. At first, 0.5-gm. charges of water-slaked and oven-dried lime were weighed on uniformly folded 9-cm. filters and enclosed in flat wire frames. Four of these were then placed upon a fallow soil surface and four were imbedded in the soil at a depth of $2\frac{1}{2}$ to 3 inches. Protection from rain was afforded without effecting any restriction in wind movement. After different periods the charges were analyzed for CO_2. This procedure was later discontinued because the filters incorporated within the soil became apparently more moist than those of the surface. Possibly the factor of greater moisture supply thus afforded to convert the hydrate from solid to liquid phase was offset by the tendency toward diminution of pore space and restricted CO_2 movement through the paper placed within the soil. The paper cartons were replaced by glass cartridges $\frac{1}{2}$ inch by $3\frac{1}{2}$ inches. One end of each cartridge was closed by a brass wire gauze and $\frac{1}{4}$ inch of quartz sand was then inserted. Enough sand to fill the cartridges to within $\frac{1}{4}$ inch was then mixed uniformly with 0.5 gm. $Ca(OH)_2$ and introduced into the glass tube. A layer of sand $\frac{1}{4}$ inch thick was next added and the end of the cartridge closed by means of a wired wire-gauze cap. These cartridges were placed upon the surface and in the soil simultaneously and subsequently analyzed for $CaCO_3$ as formerly. This procedure gave the results recorded in table 48.

The data of table 48 show that without exception the lime placed within the soil underwent carbonation more rapidly than did that exposed on the surface. In this experiment no chemical alteration was effected within the soil and no biologically accelerated or depressed CO_2 evolutions were involved. Apparently, then, if no appreciable absorption of lime by soil were to transpire and the lime incorporated within the soil were in amounts sufficiently small to activate, rather than depress biological agencies, the results would have been the reverse of those obtained in the tank and tumbler exposure experiments. This point will be further considered.

The contents of table 48 also bring out a very interesting comparison between the rapidities of carbonation which were effected in the several soils. The organic-matter content of the sandy loam and that of the stony loam were very low. The volatile matter determined on 1-mm.-mesh material from these two soils was 2.1 per cent and 2.0 per cent, respectively, as compared to 5.6 per cent from the Dunkirk silt loam. The sandy soils were of fossil delta derivation and were almost entirely devoid of clay or silt. The sandy loam is that of the quadrangle of the New York State College of Agriculture, while the stony sandy loam is located just east of Caldwell Hall. Only about one-third of the stony loam was sufficiently fine to pass the 1-mm. sieve. This soil had also been recently top-dressed with an indefinite amount of manure. The silt loam is that of the plats of Caldwell Field on the University Farm. While the total organic matter in the sands was decidedly less than in the case of silt, the freedom of diffusion of the CO_2 of the sand

TABLE 48

Comparison of the speed of carbonation of $Ca(OH)_2$ *on surface and that within fallow soils, where direct contact of lime and soil was prevented by mixing lime with sand and placing the mixture in glass tubes*

SOIL	LOCATION OF EXPOSED LIME	DATE OF EXPOSURE	PERIOD OF EXPOSURE	CHARGE EXPOSED	CARBONATION IN SEPARATE SIMULTANEOUS EXPOSURES						
					A	B	C	D	E	F	Average
			days	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Dunkirk silt loam	On surface	July 7	8	0.5	10.6	11.8	10.7	10.4	10.4	9.9	10.6
	In soil	July 7	8	0.5	20.7	21.1	17.8	18.3	18.3	18.9	19.2
Dunkirk silt loam	On surface	July 15	17	0.5	16.3	15.9	18.6	16.3	13.5	13.1	15.6
	In soil	July 15	17	0.5	32.7	33.3	38.6	38.8	39.9	31.4	35.8
Dunkirk stony sandy loam	On surface	May 31	4	0.5	7.3	5.9	6.5	6.5			6.6
	In soil	May 31	4	0.5	24.3	20.6	33.1	32.6			27.7
Dunkirk stony sandy loam	On surface	June 23	8	0.5	13.7	11.3	10.9	10.6			11.6
	In soil	June 23	8	0.5	45.1	40.5	46.5	45.8			44.5
Dunkirk sandy loam	On surface	May 2	2	0.5	46.5	46.5	49.6	42.7	41.5	48.8	45.9
	In soil	May 2	2	0.5	59.9	67.1	66.8	58.1	53.9	55.3	60.2
	On surface	May 24	2	0.5	49.5	48.5	48.2				48.7
	In soil	May 24	2	0.5	61.8	55.8	57.0	65.7			60.3

was probably greater because of larger pore space and less colloidal material to exert the phenomenon of surface adsorption of the gas.

The results of table 48 were verified by repetition with slight variation of technic, at the University of Tennessee Station Farm, during the months of August and September. In this case the glass tubes which contained the hydrate charges had an inside diameter of $\frac{13}{16}$ inch, and were $3\frac{1}{4}$ inches long. The charges of lime were mixed with asbestos or sawdust and placed in the tubes, the ends of which were closed with glass wool. The tubes which were placed in the soil were about 3 inches below the surface. The differences in the extent of carbonation resulting from the two conditions of exposure are shown in table 49. These data also indicate very forcibly that, under the seasonal conditions encountered, reasonable amounts of lime would carbonate more rapidly within the soil at a depth of about 3 inches, than upon the surface, were it not for a depression of biological activities induced by contact of lime and soil and isolation of the lime from aerial CO_2.

It thus seems probable that when amounts of lime sufficient in quantity to satisfy a soil's coefficient of absorption, and also afford carbonate residues sufficient to justify analytical work, are mixed with the soil, the following may be anticipated. A part of the lime is absorbed in neutralizing the soil's acid properties; a part is neutralized by the CO_2 of the free soil water, together with that of the atmosphere and possibly that condensed in the soil film. The remainder, if any, has a tendency, at least temporarily, to restrict or depress the further generation of CO_2 within the soil, until the excess of the lime within the soil is further, in part or in whole, converted to the carbonate. With the restricted tendency toward CO_2 generation, the excess of lime in the soil would then carbonate less rapidly than would that lying on the surface, since the latter would have more freedom of contact with the surface movement of the CO_2 of the aerial atmosphere. With regard to light applications of lime such as those most generally to be met with in practice, the following would seem to be what would be reasonably expected. The conversion to carbonate of an application immediately mixed with the soil and one left on the surface would both be very rapid and of so nearly equivalent speed as to be considered identical for all practical purposes. However, an application that would be considered as a light one on the basis of an acre 6, an acre 8, or an acre 12 inches would be proportionally heavier when stirred in with only the upper surface inch, or the surface of 2 inches. The dryness of the mulch, augmented by the presence of fine uncarbonated lime, would most probably reduce to a minimum any CO_2 generations in the mixture of lime with the upper surface. Then, if the diffusion of CO_2 from the moist underlying soil is the same to both the mulched lime and that lying on the surface, we would expect a more rapid carbonation of that left undisturbed on the surface. For, the data of table 10 show that an admixture of lime with a dry mulch exerts a protective influence and that lime so mixed reverts to the carbonate less rapidly than when left exposed.

TABLE 49

Additional comparisons as to speed of the carbonation of $Ca(OH)_2$, *when mixed with asbestos or sawdust in glass tubes and placed within and upon the surface of fallow soils, where direct contact of lime and soil was prevented;* weight of lime charge, 0.3 gm.*

SOIL	TREATMENTS APPLIED 5 YEARS PREVIOUS TO PRESENT EXPERIMENT	DATE OF EXPOSURE	CONTAINING TUBE STOPPERED WITH	CARBONATION	
				In soil	On soil surface
				per cent	*per cent*
Cookville fine sandy loam	None	August 29 to September 5	Glass wool	68.63	41.75
Crossville fine sandy loam	None	August 29 to September 5	Glass wool	54.22	31.83
Gallatin brown loam	None	September 21 to September 28	Glass wool	44.78	17.24
Jackson sandy loam	None	September 21 to September 28	Glass wool	27.19	23.03
Cumberland loam	8 tons CaO as $Ca(OH)_2$	August 15 to August 19	Cotton	33.27	19.19
Cumberland loam	16 tons of limestone	August 15 to August 19	Cotton	36.79	18.55
Cumberland loam	48 tons of manure	August 20 to August 26	Glass wool	48.31	25.14
Cumberland loam	Limestone, 16 tons; manure, 48 tons	August 20 to August 26	Glass wool	45.62	28.34
Average				44.85	25.63

* All soils well supplied with moisture.

However, there is one additional point to be considered with reference to the speed of the carbonate reaction, as it occurs in the soil, as compared to that on the surface, when the direct contact factor is eliminated. As in the case of the charges of tables 45 and 46 where access to the soil atmosphere was afforded through the perforations in the containers, thus preventing physical contact and preventing partial sterilization, the question of the differences in the moisture supply within the soil and on its surface is germane. Therefore, it seemed advisable to expose the glass-tube charges on the surface and in the soil under conditions which would insure availabe moisture to both exposures. Therefore, the following procedure was followed. Six-tenth gram charges of $Ca(OH)_2$ were mixed with dry asbestos, or sawdust, and placed in glass tubes $\frac{13}{16}$ inch in diameter and $3\frac{1}{4}$ inches long as recorded in table 49. Instead of being stoppered with glass wool as formerly, both ends of the

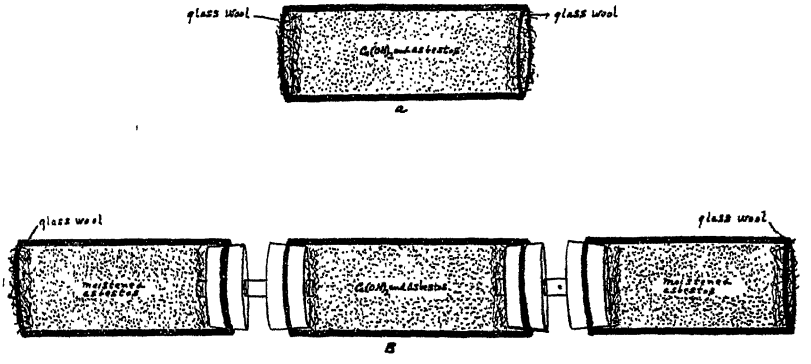

FIG. 11

a, $Ca(OH)_2$ containers used in securing data of Tables 48, 49, 51, 52 and 53.

b, Modified containers for supplying moisture to both surface and intra-soil charges of $Ca(OH)_2$, used in securing data of table 50.

tubes were closed with 1-hole stoppers. Through these was inserted a short $\frac{1}{4}$-inch glass tube, the other end of which was inserted in a 1-hole stopper and this stopper fitted into another glass tube of the same diameter as the one containing the lime. This second tube contained a porous mixture of asbestos and sawdust, which when saturated with distilled water afforded free movement of moisture and air from the atmosphere into the lime chamber. The tubes are illustrated by the sketch, figure 11, B. Working with three well moistened soils, the data of table 50 were obtained.

These data show that during fall weather the soil atmosphere will effect initially a more rapid conversion of the hydrate to the carbonate than will be effected in the case of surface exposures, where an abundance of moisture is made available to the surface charges, as well as to the charges exposed in containers within the soil. One element to be considered in this respect is that of season. Were heavy charges of hydrate exposed and extensive CO_2

fixation thus provided for, it would seem that a local paucity of atmospheric CO_2 might result and unless CO_2 diffusion to the deceminated areas should transpire, the disparity would later probably be diminished, if not reverted. These factors will be considered in succeeding paragraphs.

TABLE 50

Supplementary exposures of $Ca(OH)_2$ *in fallow soils and upon the surfaces of the same soils where moisture vapor was furnished both exposures; direct contact of lime and soil prevented by glass tube containers; weight of lime charges, 0.6 gm.*

SOIL	CONDITION OF SOIL	DATE OF EXPOSURE	CONTAINER TUBES STOPPERED WITH	CARBONATION	
				In soil	On surface
				per cent	*per cent*
Cherokee sandy loam	Wet	October 17–24	Glass wool	11.70	8.92
Cumberland loam	Wet	October 17–24	Glass wool	11.37	8.47
	Wet	October 17–24	Glass wool	10.82	8.47
Average				11.30	8.62

The factors of season and size of charges in influencing the speed of the carbonate reaction with surface and intra-soil exposures of $Ca(OH)_2$

The question as to influence of the magnitude of charge seemed essential. The additional factor of season also seemed germane, the previous exposures having been made only during warm periods. Accordingly, charges of 2 gm. of high-grade $Ca(OH)_2$ were mixed with asbestos, placed in glass cylinders and exposed on the surface and within two soils during mild winter weather experienced during the latter part of December at the Tennessee Station Farm. The procedure was then repeated, charges of 2.5 gm. of $Ca(OH)_2$ being used with five other soils. The results are given in table 51. These data show a considerable increase above the amount of absolute carbonation recorded in the case of the lesser charges of table 49, and at the same time show a reversal in the extent of the carbonation of surface exposures of $Ca(OH)_2$, as compared to those charges placed within the soil. The greater surface exposure offered by the increased charge of hydrate; more restricted CO_2 generation, because of the lower soil temperature; and decreased diffusions of soil atmosphere as a result of increased free soil water, appear as factors tending to decrease the extent of carbonation within the soil as compared to that effected in the case of the surface charges.

Additional exposures were later made. In these a simultaneous parallel between 0.6-gm. and 3-gm. charges of $Ca(OH)_2$ was secured for each of five soils, during April, at the Tennessee Station Farm. The spring season was unusually late and the soils were rather moist. The data obtained from these exposures are given in table 52. Again in every instance the surface carbonation was greater than that induced within the soil. When calculated to a

uniform basis, the results show in each instance, a greater actual carbonate formation effected by the 3-gm. charges as compared with the 0.6-gm. charges. The increases, however, are far from being proportionate. As a matter of fact, such a proportionate increase could hardly be expected, for although the amounts of hydrate mixed with the asbestos are in the proportion of 1 to 5, the area of access of soil atmosphere to hydrate is the same for each lime-container tube. On the other hand, the smaller charges may have reached a pseudo-equilibrium because of carbonate layer formations, thus minimizing

TABLE 51

Variations in the speed of the carbonate reaction obtained when excessive charges of $Ca(OH)_2$ *were exposed simultaneously on the surface and within the soil during the winter; glass containers, lime-asbestos mixtures, glass wool stoppers and no contact between soil and lime*

SOIL	DATE OF EXPOSURE	CONDITION OF SOIL	$Ca(OH)_2$ CHARGE	CARBONATION	
				Soil surface exposure	Intra-soil exposure
			gm.	*per cent*	*per cent*
Cumberland loam*.....	December 19–23	Wet	2.0	20.96	18.25
Cumberland loam.....	December 19–23	Wet	2.0	21.88	18.81
Jackson fine sandy loam.....	December 30–January 2	Rather wet	2.5	11.65	10.09
Cherokee sandy loam.....	December 30–January 2	Rather wet	2.5	11.72	11.51
Cumberland loam*.....	December 30–January 2	Rather wet	2.5	13.61	11.90
Cumberland loam.....	December 30–January 2	Rather wet	2.5	12.33	11.74
Cumberland loam.....	December 30–January 2	Rather wet	2.5	12.13	11.96
Average.....				14.89	13.47

* Each sample of Cumberland loam from a different cylinder and 5 years previously subjected to different treatments.

the effective surface and actually permitting CO_2 to be diffused through the tube without further carbonation having been effected; while the larger charge of further active surface may have absorbed this CO_2 which possibly may have diffused unabsorbed through the smaller charges.

The parallel between the 0.6-gm. and 3-gm. charges was again observed by making additional exposures after the same soil had warmed up considerably during the latter part of May. The data secured in this exposure are given in table 53. These data are in every instance the reverse in relationship to those of tables 51 and 52, but in harmony with those of tables 48, 49 and

50. While the intra-soil exposures again show a more extensive carbonation within the soil than on its surface, for both the 0.6-gm. and the 3-gm. charges, the extent of carbonation in the heavier charges is again not proportionate to the increase in amounts exposed. The relationship between the variation in amount of charges and the uniformity of exposure to soil atmosphere has been considered in the preceding paragraph.

TABLE 52

Variations in the speed of the carbonate reaction obtained with light and heavy charges of $Ca(OH)_2$ *under simultaneous surface and intra-soil exposures, during early spring; glass containers; ignited soil and lime mixtures, glass wool stoppers and no contact between soil and lime*

SOIL	DATE OF EXPOSURE	CONDITION OF SOIL	$Ca(OH)_2$ CHARGE	CARBONATION			
				Of theoretical		On uniform basis of 0.6 gm.	
				Soil surface	In soil	Soil surface	In soil
			gm.	*per cent*	*per cent*	*per cent*	*per cent*
Cherokee sandy loam	April 2–8	Moist	0.60	30.41	24.93	30.41	24.93
	April 2–8	Moist	3.00	7.42	6.81	37.10	34.05
Cumberland loam	April 2–8	Moist	0.60	31.53	25.90	31.53	25.90
	April 2–8	Moist	3.00	11.15	8.30	55.75	41.50
Jackson fine sandy loam	April 2–8	Moist	0.60	32.50	20.20	32.50	20.20
	April 2–8	Moist	3.00	11.44	4.99	57.20	24.95
Cumberland loam * (a)	April 2–8	Moist	0.60	30.18	27.61	30.18	27.61
	April 2–8	Moist	3.00	11.07	7.81	55.35	39.05
Cumberland loam † (b)	April 2–8	Moist	0.60	32.98	28.73	32.98	28.73
	April 2–8	Moist	3.00	10.54	7.58	52.70	37.90
Average				20.92	16.29	41.57	30.48

* Received 16 tons of dolomite and 48 tons of manure 6 years prior to present experiment.
† Received 16 tons of dolomite 6 years prior to present experiment.

From the survey of the data of tables 48 to 53, it appears that more extensive carbonation is effected within the soil when seasonal conditions are such as to accelerate CO_2 generation and diffusion to the extent that the restricted soil atmosphere diffusions overcome the factor of less restricted movement of the aerial atmosphere with its lesser CO_2 content. With lower soil temperatures and depressed diffusion incident to increase in free water content, the surface exposures of hydrate demonstrate a more rapid carbonation.

TABLE 53.

Variations in the speed of the carbonate reaction obtained with light and heavy charges of $Ca(OH)_2$ *under simultaneous surface and intra-soil exposures, during late spring and early summer; glass containers, quartz and asbestos mixture with lime; glass wool stoppers and no contact between soil and lime.*

SOIL	DATE OF EXPOSURE	CONDITION OF SOIL	$Ca(OH)_2$ CHARGE	CARBONATION			
				Of theoretical		On uniform basis of 0.6 gm.	
				Soil surface	In soil	Soil surface	In soil
			gm.	*per cent*	*per cent*	*per cent*	*per cent*
Cherokee sandy loam	May 19 to June 4	Optimum	0.6	32.42	68.21	32.42	68.21
	May 19 to June 4	Optimum	3.0				
Jackson fine sandy loam	May 19 to June 4	Optimum	0.6	30.81	90.09	30.81	90.09
	May 19 to June 4	Optimum	3.0	10.82	18.21	54.10	91.05
Cumberland loam*	May 19 to June 4	Optimum	0.6	31.61	85.10	31.61	85.10
	May 19 to June 4	Optimum	3.0	20.81	26.28	104.05	131.40
Cumberland loam†	May 19 to June 4	Optimum	0.6	58.24	64.51	58.24	64.51
	May 19 to June 4	Optimum	3.0	19.27	21.94	96.35	109.70
Average				29.14	53.48	58.22	91.44

* Received 30 tons of manure 6 years previous to present experiment.

† Received 16 tons of dolomite and 48 tons of manure 6 years previous to present experiment.

DOES BURNT LIME DERIVE CO_2 FOR ITS CARBONATION BY CHEMICAL ACTION UPON SOIL ORGANIC MATTER?

The statement that caustic lime "burns up the humus" of a soil is commonly heard in agricultural discussion and is to be found in many writings. The belief is prevalent that a deleterious action upon soil organic matter follows the use of caustic lime, while no such result is to be attributed to the use of ground limestone. Two viewpoints are maintained, relative to the action of lime on soil organic matter. By one group it is held that the action of lime, in the assumed depletion of organic matter, is primarily a chemical function. The following citations are typical. Richardson (56) believes, "Its destructive action is due to its caustic character. It rapidly destroys humus, and thus tends to rob the soil of its most valuable constituent." Reinecke (55) writes, "The very fact, however, that they are alkaline and hence caustic makes their extensive use dangerous since caustic lime (lime in oxide and hydrate form) destroys soil humus." It might well be noted at this point that shippers of hydrated lime experience no difficulty, when using paper bags as containers for the water slaked product. Hopkins (36) teaches

that precautions should attend its use for, "in other words the use of lime, if continued tends rapidly to exhaust the soil and ultimately to leave it practically ruined." It is interesting to note in this connection that such an observation was made following the use of marl, in 1856, by Kitchell (40), who noted almost complete sterility for a period of 18 years following the use of excessive amounts of marl. The sterility, however, disappeared upon the use of barnyard manure. To quote, "On the property of Mr. James H. Struble in Sandiston Township, is a barren spot of land to which marl, or calcareous sinter, was applied 18 years ago. Since this time, as I was informed, no vegetables would grow except when a heavy coat of barnyard manure or vegetable matter is applied to it and then but one luxuriant crop can be obtained." The character of the soil was not specified. It is possible that such an observation may be accounted for by the use of the toxic marls to be found in that locality. Dr. Hopkins (36) also cites Webster as defining *caustic* as being "capable of destroying the texture of anything or eating away its substance by chemical action." He then states, "This definition well describes the action of caustic lime upon the organic matter of the soil." It might here be stressed that the inherent and characteristic activity of caustic or burnt lime is due to its affinity for *water*. This affinity is readily satisfied by moisture available from adjacent soil and aerial atmosphere, when burnt or unslaked lime is lying upon the soil surface. Subsequent to the taking up of moisture and conversion of the oxide to the hydrate, neither the solid phase of this later nor the solution phase of hydroxide appears to exert any destructive action on soil organic matter. This may be deduced from the data derived from experiments involving contact of both dry and moist soil with the solid hydrate (table 54), and the continued contact of the concentrated hydroxide solution with the several soils as given in table 55.

Christie and Martin (10) studied the effect of a 0.4 per cent application of CaO on an alkaline soil, by examining the concentration of the soil extract 24 hours after incorporation of the lime. They found a marked increase in the amount of calcium, magnesium, potassium and phosphorus, with a decrease in nitrates. In discussing their results they state, "It is a well known fact that lime decomposes organic matter in soils and, therefore, it might well be contended that the increases in total solids were due to soluble organic matter. In case of about half of the soils examined, the electrical conductivity of the soil extract was measured. The figures obtained were in direct relation to the total-solids determination, showing that at least the greater part of the increases were due to inorganic and not to organic matter."

By another group, it is contended that any decrease in soil organic matter, following the use of burnt or hydrated lime, is attributable to the acceleration of biological activities; and hence that the judicious use of lime is no more to be discriminated against than is soil cultivation, or fertilizer stimulation, both of which also effectuate the same result. The more conservative view, that there is lacking proof to support the contention that burnt lime effects

chemical destruction of soil organic matter under conditions of field practice is held by Frear (22), Wheeler (70, 71) and Fippin (17). While numerous experiments could be cited to demonstrate the accepted truth as to acceleration of biological activities following the use of lime, no differential studies have been put forward to demonstrate separately the action to be attributed to chemical and biological factors.

The data most generally utilized in an attempt to substantiate the claim that CaO will, by chemical action, deplete the soil organic matter are those reported by Frear (20) in 1900. In this work, which was done 18 years after the inauguration of the experiment, the limed plats toward the west end of the tiers are compared with a composite of all the check plats, some of which are at least one-tenth mile distant. The comparisons were made and the conclusions were drawn on the assumption that initially the plats were of identical or very comparable composition. Dr. Frear has since showed that there exists a marked dissimilarity in the present composition of different portions of the driveways between the tiers. These driveways have been continuously in grass, since the inauguration of the experiment. The limed plats were analyzed 12 years later by the writer (47). In this case, however, the basis of comparison was a composite of the four check plats immediately adjacent to the plats receiving lime alone and between these and the plats treated with ground limestone. These analyses emphasize one comparison as pertinent. In 1899, Hess found by furnace combustion of 0.5-mm. composite samples of the five quadruplicate series of untreated plats 2.830 per cent organic matter as compared to 2.530 per cent from a composite of the four burnt lime plats. On the other hand in 1912 the writer analyzed 100-mesh composites of the four untreated plats immediately adjacent to the four plats receiving burnt lime alone, sampled simultaneously in the same manner. These later analyses give 2.555 per cent organic matter for the untreated soil as compared to 2.529 per cent for the burnt lime plats. Comparing the samplings of the four limed plats as obtained by Hess and again by MacIntire, 12 years later, it appears that the burnt lime plats have lost but 0.001 per cent of organic matter. On the other hand if the composite of the four untreated plats immediately adjacent to the lime plats is considered as comparable to the composite of all untreated plats, as sampled by Hess, then the *untreated plats have lost 0.275 per cent of organic matter*. It is certainly as consistent to compare the average composition of all the check plats at one period, with the average composition of the four check plats adjacent to the lime plats at another period, as it is to compare the average composition of all the check plats with the average composition of the four limed plats at corresponding periods.

On the assumption of uniform original composition, now established by Dr. Frear as untenable, a slightly less organic matter content, 0.026 per cent, is found in the CaO-treated soil than is to be found in the limestone-treated plats. The difference is small, however, and is well within the error of careful

sampling and analysis, as may be seen from the compositions of the eight burnt-lime-treated plats (table 9). Eliminating from consideration the question of error of sampling, there remains the additional undetermined factors, namely, variation in influence upon biological activities and *differences in the amounts of root stubble incorporated in the two series of lime-treated plats.* More recently, however, Dr. Frear has discussed the matter comprehensively in the light of the more recent data. From a comparison between the data of Hess and the above-cited analysis by the writer, Dr. Frear (22) justifies his conclusion that data from the general fertilizer plats of the Pennsylvania Station do not warrant the statement that organic matter has been destroyed by chemical action of the quick lime. He further stated that his original data had been utilized commercially as the basis of a limestone propaganda to an extent further than he felt was justified.

There remains an additional undetermined factor, i.e., the possibility of difference in the incipient action of lime upon the original soil organic matter and that exerted upon the residues from the initial organic matter and the organic matter incorporated from stubble since the end of the 18-year period.

In addition to the data reported by the Pennsylvania Station analyses obtained from plats which had been limed for a period of ten years have been reported by Bear (3) of the West Virginia Station. In this case the soil was sampled at the beginning of the experiments, and the question of variation due to sampling also was considered. Bear concluded that the application of burnt lime is more conducive to the destruction of soil organic matter than is limestone.

In carefully checked plat work, Mooers (53) has concluded from determinations of ammonia-soluble matter that the use of 1800 pounds of burnt lime per acre has slightly decreased the more active humus as compared to the untreated plats. In experiments with imbedded pots, Wheeler, Sargent and Hartwell (72) also have reached the same conclusions. It is interesting to note, however, that phosphates and other fertilizers devoid of destructive characteristics effectuated the same result. This is a strong indication that acceleration of biological activities may account for the decrease in active organic matter. Other contributions on the subject are those of Hartwell and Kellog (29), Ritter (57), Andre (2) and Densh (11). The latter writer obtained evidence of ammonia formation from contact between lime and peat, vegetable mould, etc., after an 82-day period of contact. No such duration of uncarbonated lime could occur, however, under normal field conditions. It is, of course, conceded that nitrogen may be split off as NH_3 from organic forms, by hydrolytic processes induced by $Ca(OH)_2$. However, if the lime is mixed in the soil, the soil's absorption power would inhibit loss of NH_3 as gas. This was demonstrated in the case of the experiment embodied in table 54, in which instance no NH_3 could be aspirated off. In combination with water this would certainly not be leached any more rapidly than would nitrates, the production of which lime and other favorable agencies

are known to accelerate. Furthermore, admitting the doubtful hypothesis that complete carbonation would not transpire prior to harrowing in of applied lime, any NH_3 set free chemically within the soil would quickly undergo change to ammonium carbonate or urea and probably nitrification as a result of stimulated biological activities. But, marked increase in ammonification, as a result of partial sterilization, has been shown by Russell and Hutchinson (59) to be probably the primary cause of increased fertility attendant upon the sterilizing treatment. In light of the above facts, any chemical ammonification induced by burnt lime within the soil assumes the rôle of an argument for, rather than against, its use.

It is often stated, with possible justification, that *simultaneous* surface applications of manure and burnt lime should not be made, because of the loss of ammonia or ammonium carbonate by volatilization. There would appear to be no particular reason why such applications should be made simultaneously. Granting that lime will accelerate loss of ammonia, when lime and manure are applied to the surface at the same, or approximately the same time, it should be remembered that we have no experimental evidence upon which to base the assumption and also that ammonia losses from manure freshly applied to the surface in the field are also to be sensed *in the absence of lime.*

The chemical destruction of organic matter by lime would involve the liberation of CO_2 which would be partly or completely absorbed by the $Ca(OH)_2$, thus hastening its conversion to $CaCO_3$. Hopkins (36) states, "The lime breaks down the organic compounds and unites with the liberated carbon dioxide or other acid products," and "Caustic lime is not only a powerful agent in hastening the destruction of organic matter . . . . " Again, "Thus there are two principal effects produced by applying lime to soils: one . . . . and the other is a more active decomposition or destruction of the soil itself, especially of its organic matter or humus content." It is, then, of interest and of importance to determine whether the applied lime derives CO_2 required for carbonation partly from chemical action of lime on organic matter, as well as from atmosphere, soil atmosphere and accelerated biological activities. An attempt was made to obtain data on this phase of the question.

The following plan was adopted: Fifty-gram charges each of two calcareous soils, the brown loam and the grey silt loam of the mulch exposure tests of table 10 previously described, were weighed into 300-cc. Erlenmeyer flasks. These were provided with two-hole rubber stoppers, through which were inserted cotton-plugged glass tubing with Geissler stop-cocks attached. Each treatment and check was made in triplicate. To each flask was added a 0.4-gm. charge of high-grade $Ca(OH)_2$. The two soils used were from basket experiments and two years previously had received treatments of precipitated $CaCO_3$ equivalent to 9000 pounds of CaO in excess of the "lime requirement" indicated by the Veitch method. In this instance

absorption of the applied hydrate was minimized and most, if not all, of the charge of caustic lime left free to exert any possible chemical activities upon organic matter. The condition to which the triplicate charges of each soil were submitted were dry, both sterile and non-sterile, and wet, both sterile and non-sterile. The dry soil was sterilized in an autoclave by subjection to 15 pounds of steam pressure for 30 minutes. Soil, hydrated lime, and distilled water for soil moisture were sterilized separately and mixtures made of the sterile substances. It should not be assumed that powdered hydrated lime is absolutely sterile, *per se*. At the request of the writer, Dr. J. K. Wilson, of Cornell University, made several plate cultures from bottled samples of the freshly slaked, dry, hydrated lime used by the writer in the tumbler-exposure experiments and he obtained a vigorous growth of fungi.

TABLE 54

Analyses made to determine amounts of any CO_2 liberated as a result of contact of 0.4 gm. $Ca(OH)_2$ and 50 gm. of soil in closed flasks under dry and moist conditions, both sterile and non-sterile; time, 126 days

SOIL	TREATMENT	CO_2 IN SOIL AND ATMOSPHERE OF FLASKS	CO_2 IN BLANKS	CO_2 LIBERATED CHEMICALLY OR BIOLOGICALLY FROM ACTION OF LIME ON SOIL	
				Gm. from 50 gm. of soil	Per cent
		gm.	*gm.*		
Cumberland loam	CaO dry and sterile	0.0622	0.0546	0.0076	0.015
	CaO dry and non-sterile	0.0706	0.0614	0.0092	0.018
	CaO wet and sterile	0.0681	0.0731	0.0050	0.010
	CaO wet and non-sterile	0.0976	0.0989	0.0013	0.003
Silty loam	CaO dry and sterile	0.0203	0.0211	0.0008	0.002
	CaO dry and non-sterile	0.0256	0.0249	0.0007	0.001
	CaO wet and sterile	0.0410	0.0372	0.0038	0.007
	CaO wet and non-sterile	0.0592	0.0501	0.0091	0.018

Subsequent to admixtures of dry soil and $Ca(OH)_2$, 7.5 cc. of water was added to each of the 50-gm. charges which were to be kept moist. After mixing, the flasks were freed of their atmospheric CO_2, closed and kept in a darkened closet without further disturbance. At the end of 48 days single determinations of CO_2 from checks and each triplicated treatment were made as a guide for future analytical work. No activity of lime having been noted, the remaining duplicate flasks were left for a total period of 126 days. At the end of this period the flasks were aspirated to absolutely insure the absence of gaseous CO_2. Possible presence of free NH_3 also was considered and the aspirated air tested therefor, with negative results. If any ammonia was liberated by the treatment, it remained absorbed by the soil. Carbonate determinations were then made on the 50-gm. charges of soil. The averages

of duplicate determinations are given in table 54. These analyses show that no positive chemical decomposition ensued as a result of long continued contact of soil and lime. While an appreciable increase in $CaCO_3$ might be expected to occur from fixation by the lime of any CO_2 liberated through enhanced biological evolution, it appears from the data that the charge of lime was sufficiently large to insure enough of $Ca(OH)_2$ in solution to effect partial or complete sterilization. These conditions were much more severe and were extended over a much longer period than would ever be encountered in practice. No field conditions would ever effect such long continued contact between undissolved $Ca(OH)_2$ and moist soil. For, as has been shown, carbonation is relatively rapid in near-surface applications in reasonable quantities. Neither would there occur in practice such extensive admixture of soil and treatment; nor would there obtain such extensive surface contact of soil and dissociated hydrate during the period prior to its carbonation under practical conditions.

If no positive decomposition of organic matter is effected chemically by lime under the conditions maintained in this experiment, it is difficult to assume that lime under field conditions would accomplish such a result.

Effects produced by contact of saturated $Ca(OH)_2$ *solution and soil*

The question was still further considered by subjecting the soil to possibly even more severe treatment. Quadruplicate samples of a number of soils were weighed into 300-cc. Erlenmeyer flasks which were then stoppered with cotton and sterilized by overnight heating at 105°C. in an electric oven. Two of the charges were then freed from the CO_2 of their atmosphere and agitated in closed flasks for 3- and 4- and 15-hour periods, with 50 cc. of saturated lime-water containing 0.0596 gm. of CaO, or 0.238 per cent, 0.298 per cent and 0.596 per cent, respectively, for the 25-, 20- and 10-gm. soil charges. These amounts would be equivalent to 4760 pounds, 5960 pounds and 11,920 pounds, respectively, of CaO per acre 2,000,000 pounds of soil. The two remaining charges were treated in the same manner, except that CO_2-free distilled water was substituted for the lime-water. After vigorous and continued agitation for the specified periods the carbonate CO_2 was determined by liberation through additions of 20 cc. of 1 to 1 HCl. The results are given in table 55.

Inspection of these data shows that, within laboratory error, attendant upon very careful manipulation, no difference was obtained between the treatments of distilled water and lime-water. With four of the ten soils, negative results were obtained. Two gave indications of increase less than 0.0025 per cent; three gave apparent increase less than 0.0175 per cent, while one alkaline muck gave 0.0270 more CO_2 for the lime-water. The high percentage of organic matter in the muck soils would certainly appear to afford conditions calculated to permit any destructive action by the lime-water, especially when the latter was present at the rate of over 11,000 pounds of

TABLE 55

Nugatory action resulting from agitated contact of sterile soils and saturated water solution of $Ca(OH)_2$ in closed flasks—possible decomposition of organic matter anticipated and allowed for by absorption of liberated CO_2 in $Ca(OH)_2$ with subsequent determinations of combined CO_2 as compared with parallel blanks of CO_2-free distilled water

SOIL	REACTION TO LITMUS	VOLATILE MATTER	CHARGE	TREATMENT	PERIOD OF AGITATED CONTACT	CO_2 FOUND		CO_2 LIBERATED FROM SOIL BY CONTACT WITH $Ca(OH)_2$	
		per cent	*gm.*		*hours*	*gm.*	*per cent*	*gm. per charge*	*per cent*
Volusia silt loam	Acid	10.7	25	$Ca(OH)_2$	3	0.0069	0.0276	0.0006	0.0023
	Acid	10.7	25	H_2O	3	0.0063	0.0253		
Dunkirk clay loam	Alkaline	5.2	25	$Ca(OH)_2$	15	0.0441	0.1764	0	0
	Alkaline	5.2	25	H_2O	15	0.0442	0.1768		
Fine sandy humus loam	Neutral	12.1	25	$Ca(OH)_2$	4	0.0286	0.1144	0.0027	0.0108
	Neutral	12.1	25	H_2O	4	0.0259	0.1036		
Volusia silt loam	Acid	7.8	25	$Ca(OH)_2$	3	0.0055	0.0220	0	0
	Acid	7.8	25	H_2O	3	0.0055	0.0220		
Volusia fine sandy loam	Acid	15.3	25	$Ca(OH)_2$	4	0.0154	0.0615	0.0031	0.0123
	Acid	15.3	25	H_2O	4	0.0123	0.0493		
Genessee silt	Alkaline	12.2	25	$Ca(OH)_2$	3	0.0110	0.0440	0	0
	Alkaline	12.2	25	H_2O	3	0.0110	0.0440		
Virgin Genessee silt	Alkaline	14.3	25	$Ca(OH)_2$	3	0.0125	0.0500	0.0002	0.0008
	Alkaline	14.3	25	H_2O	3	0.0123	0.0492		
Muck	Alkaline	67.0	20	$Ca(OH)_2$	3	0.0924	0.4620	0	0
	Alkaline	67.0	20	H_2O	3	0.0924	0.4620		
Muck	Acid	94.8	10	$Ca(OH)_2$	3	0.0059	0.0590	0.0017	0.0170
	Acid	94.8	10	H_2O	3	0.0042	0.0420		
Muck	Alkaline	67.8	10	$Ca(OH)_2$	3	0.0489	0.4890	0.0027	0.0270
	Alkaline	67.8	10	H_2O	3	0.0462	0.4620		
Average									0.0070

CaO per acre under such exaggerated conditions of contact. The results, however, could not be considered as absolutely proving the decomposition of organic matter by hydrated lime, even if the several minute plus differences were taken as positive indication of chemical activities. For, it is quite possible that some substances of acid character might be precipitated by the lime, thus affording substances more easily decomposed by the acid used to liberate combined CO_2. Then, too, in the mineral soils some potassium exchange would be expected to insure, and this strongly dissociated base might effect, slight decomposition of organic matter.

It is apparent that under the sterile intensified experimental conditions there is exhibited no loss of organic carbon from the action of hydrated lime. It is, therefore, difficult to believe that there occurs in the field any *chemical decomposition* of carbonaceous material as a result of contact between soil and caustic lime.

Field cylinder studies relative to the comparative activities of CaO, $Ca(OH)_2$ *and finely divided* $CaCO_3$ *upon initial and supplementary soil organic matter*

The question of the chemical decomposition of soil organic matter, either that of initial stores or that applied was, in tables 54 and 55, considered solely as the basis of the relationship of such a possible decomposition to the generation of CO_2 which would influence the speed of the reversion of hydrate to carbonate. However, the exposed cylinder experiments used in the carbonation studies afforded an opportunity for the determination of any difference in the residual organic matter subsequent to the use of burnt lime as compared to that residual from chemically equivalent carbonate checks. No brief is offered for burnt or hydrated lime as compared to ground limestone, nor is consideration given to the comparative virtues or commercial values as related to their use in practice. The object sought was to ascertain whether it is true that burnt or hydrated lime effects *chemical disintegration* and decrease of soil organic matter. As previously pointed out, the hydrate and oxide applications may be considered as identical, since there was an abundance of moisture in the soil at the time of incorporation, and this moisture quickly effected a hydration of the oxide. It should be stressed that the experimental conditions imposed in the cylinder studies were much more severe than would be those encountered in agricultural practice. The lime treatments of the cylinder experiments were mixed throughout the upper 6 inches of the 8 inches of soil placed in each cylinder. Thus the added CaO and $Ca(OH)_2$ had extensive contact with the soil before reversion to carbonate could take place. On the other hand, it has been shown that quick carbonation ensues when lime is left exposed on the soil surface, and hence in practice a large part, it not practically all, of the applied lime will be incorporated with the soil as the carbonate, rather than as the hydrate, provided the lime is spread in fine condition.

It is obvious that even with ground limestone of unusual fineness there would be a considerable disparity between the surface exposure of such particles and that of particles of carbonate formed from applied oxide and hydrate. Such a differential in surface exposure would mean a variation in the alkalinity of the soil medium as induced by difference in amounts of lime dissolved by the soil solution and absorbed by the soil. Therefore, pure precipitated carbonate devoid of hydrate, rather than limestone was used as a control. The soil used was thoroughly mixed, screened, and remixed, and it was assumed that each 200-pound lot of soil placed in each 1/10,000-acre cylinder was of the same composition initially. Accordingly, after the speed of carbonation studies had indicated complete carbonation of the applied lime, samples were taken for the determination of total residual organic matter. The determinations were made by the wet combustion method as modified by Schollenberger (61). The absorption of carbonate CO_2 liberated by acid, and that engendered by the oxidation process was accomplished by means of NaOH contained in elongated absorption towers. These were filled with alternating pockets of solid glass rods and glass beads. The absorption solution was washed from the towers into 500-cc. graduated flasks. Barium chloride was added and the solutions made to mark and agitated. After the $BaCO_3$ had settled, 200-cc. aliquots of the clear supernatant hydrate were titrated, phenolphthalein being used as an indicator.

Discussion of the results of tables 56 and 57

In considering the results of tables 56 and 57 it should be remembered that, disregarding the flask-container studies of tables 54 and 55, any variation in the residual organic matter of these field cylinders might be attributed to combined chemical action and biological activation induced by the treatment, or solely to either agency, according to the viewpoint assumed. But, the data of tables 54 and 55 demonstrate that there occurs no positive disintegration of organic matter involving the liberation of CO_2. However, it is possible that some organic matter may be so changed as to render it more susceptible to oxidation by biological activities or by an enhancement of the inherent oxidative properties of the soil. In considering the activities of CaO and $Ca(OH)_2$, it is only the immediate effect with which we are concerned, that is, the effect produced up to the time of complete conversion of hydrate to carbonate. For, after the conversion of the hydrate to the carbonate, it is then a question of the activation induced by finely divided $CaCO_3$. Hence, a comparison between the amount of soil organic matter remaining at the end of a long period subsequent to treatments of CaO or $Ca(OH)_2$ and that residual after a treatment of ground limestone would be analogous to a comparison between the effect of pulverulent $CaCO_3$ and coarser ground limestone.

As has been pointed out, the analytical results offered by Frear and those obtained by the writer in studies of the Pennsylvania Station plats are to be

considered in the light of the fact that we have no accurate knowledge as to the amounts of plant residues turned under in either the limestone or the burnt lime plats. But in the case of the cylinder experiments there was no interference from varying crop residues, and such incorporations of organic

TABLE 56

Total organic matter residual in Cumberland loam soil of field cylinders subsequent to incorporation of separate applications of CaO, $Ca(OH)_2$ *and* $CaCO_3$, *on the basis of chemical equivalence to 4,000 pounds of* CaO *per 2,000,000 pounds of soil; with and without supplementary applications of manure*

LABORATORY NUMBER	RIM NUMBER	SERIES	DATE OF SAMPLING 1913	TREATMENT		CARBONATE CO_2	TOTAL CO_2	ORGANIC CO_2 MOISTURE-FREE BASIS	AVERAGES		
				Lime	Manure per acre				Number of cylinders sampled	Number of analyses	Organic CO_2
					tons	*per cent*	*per cent*	*per cent*			*per cent*
2692	1	M	July 28	CaO	None	0.00	5.22	5.22	2	5	5.28
2697	1	L	August 11	CaO	None	0.01	5.34	5.33			
2693	2	L	August 11	$Ca(OH)_2$	None	0.02	4.96	4.94	2	5	5.02
2709	2	M	August 11	$Ca(OH)_2$	None	0.01	5.12	5.11			
2694	3	L	August 11	$CaCO_3$	None	0.02	4.94	4.92	2	4	5.07
2710	3	M	August 11	$CaCO_3$	None	0.01	5.23	5.22			
2711	7	M	August 11	CaO	12	0.00	5.55	5.55	2	4	5.57
2940	7	L	September 15	CaO	12	0.00	5.58	5.58			
2696	8	L	August 11	$Ca(OH)_2$	12	0.00	5.57	5.57	2	4	5.54
2960	8	M	September 15	$Ca(OH)_2$	12	0.00	5.51	5.51			
2922	9	L	August 15	$CaCO_3$	12	0.02	5.30	5.28	2	5	5.40
2931	9	M	August 15	$CaCO_3$	12	0.00	5.52	5.52			
2698	13	L	August 11	CaO	30	0.02	5.68	5.66	2	4	5.69
2714	13	M	August 11	CaO	30	0.00	5.71	5.71			
2699	14	L	August 11	$Ca(OH)_2$	30	0.02	5.78	5.76	2	5	5.67
2715	14	M	August 11	$Ca(OH)_2$	30	0.00	5.68	5.68			
2925	15	L	August 15	$CaCO_3$	30	0.02	5.61	5.59	2	4	5.58
2934	15	M	August 15	$CaCO_3$	30	0.02	5.58	5.56			

matter as were made were of uniform character and of definite extent. The assurance of the necessary uniformity of initial composition was much more nearly approached in the cylinder experiments than is possible in the case of the Pennsylvania plats. For, in the latter case, the usual interpretation

which has been placed upon the chemical results from those plats is based upon the assumption of initial uniformity of composition in the area embraced by the plats. *That area is 18 acres in extent.* Even were it not for the results of Frear, which have demonstrated such an hypothesis as erroneous, one conversant with the topography and soil-type variations of those plats would know such an assumption to be untenable. It remained to be determined whether the accuracy possible in the handling of cylinder experiments

TABLE 57

Total organic matter residual in Cumberland loam soil of field cylinders subsequent to incorporation of separate applications of CaO, $Ca(OH)_2$ *and* $CaCO_3$ *on the basis of chemical equivalence to 16,000 pounds of* CaO *per 2,000,000 pounds of soil; with and without supplementary applications of manure*

LABORATORY NUMBER	RIM NUMBER	SERIES	DATE OF SAMPLING 1913	TREATMENT		CARBONATE CO_2	TOTAL CO_2	ORGANIC MOISTURE-FREE BASIS	NUMBER OF CYLINDERS SAMPLED	NUMBER OF ANALYSES	ORGANIC CO_2
				Lime	Manure per acre						
					tons	*per cent*	*per cent*	*per cent*			*per cent*
2704	25	L	August 11	CaO	None	0.43	5.36	4.93	2	4	4.98
2720	25	M	August 11	CaO	None	0.45	5.47	5.02			
2705	26	L	August 11	$Ca(OH)_2$	None	0.42	5.33	4.91	2	4	4.93
2721	26	M	August 11	$Ca(OH)_2$	None	0.44	5.38	4.94			
2706	27	L	August 11	$CaCO_3$	None	0.69	5.63	4.94	2	5	5.03
2691	27	M	July 28	$CaCO_3$	None	0.54	5.66	5.12			
2701	19	L	August 11	CaO	48	0.43	5.99	5.56	2	6	5.46
2717	19	M	August 11	CaO	48	0.41	5.77	5.36			
2702	20	L	August 11	$Ca(OH)_2$	48	0.47	6.07	5.60	2	4	5.57
2718	20	M	August 11	$Ca(OH)_2$	48	0.53	6.07	5.54			
2928	21	L	August 15	$CaCO_3$	48	0.67	6.23	5.56	2	7	5.63
2973	21	M	September 15	$CaCO_3$	48	0.54	6.24	5.70			

was of such extent as to register definitely any changes induced by the lime treatments or whether it was necessary to depend upon the smaller and more accurately controlled laboratory experiments.

The 2-ton-treatment results are given in table 56 while those derived from the 8-ton cylinders are embodied in table 57. The 2-ton treatment of CaO gave an average of 5.28 per cent organic CO_2, while the two $Ca(OH)_2$-treated cylinders gave an average of 5.02 per cent. These may be considered as being four identical treatments, giving an average of 5.15 per cent against

an average of 5.07 per cent for the two carbonate checks. The difference obtained by this comparison of averages is within the difference represented by the variations between maximum and minimum of the duplicates in each pair of treatments and checks. Again, the average of the four CaO and $Ca(OH)_2$ cylinders which received simultaneous application of manure at the rate of 12 tons per acre, amounted to 5.56 per cent organic CO_2, as against 5.40 per cent for the carbonate checks; while the four caustic treatments supplemented with manure at the rate of 30 tons per acre yielded an average of 5.68 per cent organic CO_2, as against 5.58 per cent for the carbonate and manure checks. These data might be construed as indicating a partial retardation of biological activities as the result of the incorporation of the 2-ton treatments of the caustic forms of lime, when compared to the activities resultant from the equivalent applications of carbonate. However, since the data of tables 11 to 16 show practically complete absorption of both oxide and hydrate, as well as carbonate, within 10 days after their incorporation, it would seem more reasonable to assume that the differences are within the limits of error induced by mixing, sampling and analysis. And furthermore, the data of tables 17 to 21 do not tend to support the viewpoint of depression induced by the oxide and hydrate, for in the cylinders yielding the data of these tables, the rate of application was four times that of the lighter applications on the 2-ton basis.

The average residual of initial organic CO_2 in the four 8-ton oxide and hydrate cylinders recorded in table 57 amounts to 4.96 per cent, this being a composite of an average of 4.98 per cent of CaO and an average of 4.93 per cent for $Ca(OH)_2$ treatments, as against 5.03 per cent for the $CaCO_3$ blanks, one of which contained 4.94 per cent, while the other gave a percentage of 5.12. Inspection of the results secured from the 8-ton treatments of burnt lime, hydrate and carbonate, each supplemented by manure, shows an organic CO_2 occurrence of 5.56, 5.60 and 5.56 per cent, respectively, in Series L, as against a respective content of 5.36, 5.54 and 5.70 per cent for the corresponding cylinders in Series M. The average of the results from the four CaO and $Ca(OH)_2$ cylinders of both series amounts to 5.52 per cent as against 5.63 per cent for the carbonate checks.

In a joint consideration of the data of the two tables, consistency demands that the greater difference obtained in the data derived from the 2-ton treatments of CaO and $Ca(OH)_2$ as compared to 2-ton-equivalent $CaCO_3$ treatments be viewed in the light of the reversal obtained in the comparison of the three forms when applied at the rate of 8-ton equivalence. Thus the plus differences of 0.08, 0.16 and 0.10 per cent, yielding an average plus difference of 0.11 per cent, accredited to the oxide and hydrate treatments as a summation of the 2-ton treatments from the no-manure, 12-ton-manure and 30-ton-manure cylinders, respectively, is opposed to the averages minus difference of 0.09 per cent obtained by averaging the minus differences of 0.07 and 0.11 per cent obtained from the 8-ton treatments in the no-manure and 48-ton-manure cylinders, respectively.

The data of tables 56 and 57 are certainly not out of harmony with those of tables 54 and 55, and together with them demonstrate that, in the case of the several soils studied, no chemical disintegration or destruction of organic matter results from such surface applications or incorporations of lime as would be expected in practice.

SUMMARY AND CONCLUSIONS

1. The development of the practice of liming and the need of more dependable data are pointed out.

2. A résumé is given of the available data relative to the reversion of burnt and hydrated lime to the carbonate in the atmosphere; while only opinions, instead of data, are available as to the carbonation ensuing subsequent to the application of lime to soils.

3. In preliminary chemical studies, without introducing the factor of the influence exerted by soils, it was demonstrated in tables 1 to 8, that:

a. Dry CaO and dry CO_2 do not react.

b. Dry $Ca(OH)_2$ and dry CO_2 do not react.

c. Blasting of high-grade lime, relatively free of silica, did not depress its hydrating or carbonating tendencies.

d. Eight 0.3-gm. charges of re-heated hydrated lime effected a more rapid reversion to the carbonate than did an equal number of charges of the original hydrate, when the sixteen charges were simultaneously exposed to normal humidity in outdoor October weather.

e. The same proved true with fourteen charges each of original and re-burnt hydrate exposed during the month of June, charges of from 0.3 gm. to 2.4 gm. having been used.

f. In June and July exposures of high-grade CaO and $Ca(OH)_2$ derived from slaking the same CaO, the CaO effected a more rapid reversion to carbonate in the case of the light charges; the reverse was true in the case of the heavy charges.

g. The film moisture from a dry glass container-tube was sufficient to initiate the carbonate reaction.

h. Complete hydration of CaO resulted from an overnight period of contact with moist air.

i. The formation of carbonate from $Ca(OH)_2$ is controlled by the amount of free water available to convert the $Ca(OH)_2$ from solid to solution phase.

j. The reversion of CaO to $CaCO_3$ must be preceded by the hydration and solution of the CaO; but when moist CO_2 is present, the reactions are so rapid as to be considered as simultaneous.

k. A protecting film-coating of $CaCO_3$, around included nuclei of $Ca(OH)_2$, is so effective as to retard further measurable carbonation in atmospheric exposure until the film surface is disrupted.

4. The question of the continuation of lime, as the hydrate, in soils was found to be a mooted one. As bearing on this point, the lime plats of the

Pennsylvania Station were analyzed and each of the eight plats was found to be free of uncarbonated lime (table 9).

5. In soil-surface carton-container exposures, without direct contact between soil mulch and underlying moist soil, applications of $Ca(OH)_2$ carbonated more rapidly on the surface of a dust mulch, than within the mulch. Equilibrium was attained before complete carbonation was effected. Grinding of the treatments in equilibrium was followed by further carbonation (table 10).

6. In a total of one hundred and twenty-eight 1/10,000 acre field-placed cylinders, a number were used for the purpose of studying the carbonate reactions in soils. Equivalent amounts of CaO and $Ca(OH)_2$ were used against precipitated $CaCO_3$ as a check over a period during which no leaching rains fell. In some instances manure also was included (pl. 1).

7. In the field cylinder studies, maximum carbonation of 2-ton CaO and $Ca(OH)_2$ applications ensued within 5 days after the admixture of the lime with the upper 6 inches of an 8-inch depth of surface soil; while complete absorption occurred within 10 days (tables 11 to 16, and fig. 2).

8. No difference could be observed between the activities of CaO and those of $Ca(OH)_2$ in the field cylinder soils (tables 11 to 21).

9. The addition of water, simulating rainfall, hastened both carbonation and subsequent absorption by the soil of applied CaO and $Ca(OH)_2$ (tables 11 to 17).

10. The carbonate reversion apparently reached its maximum before chance was afforded for its acceleration by CO_2 generated from supplementary treatments of 12, 30 and 48 tons of dry manure (tables 12 to 20).

11. Maximum carbonation of unabsorbed CaO, from both oxide and hydrate, occurred after contact extending over a period of between 19 and 38 days in the case of the 8-ton-per-acre treatments in the cylinders (tables 17 to 21).

12. The amounts of residual $CaCO_3$ derived from the unabsorbed portions of the applied CaO and $Ca(OH)_2$ were practically the same at the end of 38-, 47- and 61-day periods, in the case of the 8-ton treatments. Again supplementary manure treatments caused no determinable acceleration in the formation of $CaCO_3$ (tables 17 to 21).

13. Approximately 96 per cent of theoretical carbonation was attained from the unabsorbed residues of 8-ton chemically equivalent applications of CaO and $Ca(OH)_2$ which were made to a moist acid loam soil, precipitated $CaCO_3$ treatments having been utilized as checks (table 21).

14. The small differences in the amounts of $CaCO_3$ residual from applied CaO and $Ca(OH)_2$ as compared with the $CaCO_3$ checks might be accounted for by assuming a consistent analytical error, or the possible occlusion of $Ca(OH)_2$ nuclei. However, the abundance of soil moisture, especially in the cylinders receiving supplementary wettings, would minimize the latter possibility. These data and similar results from the lysimeters point to a slightly greater absorption from the more soluble hydrate during the period prior to its carbonation.

15. The analytical error to be expected in making CO_2 determinations upon moist soils was shown by periodic analyses of checks (table 22).

16. In an attempt to study the speed of the carbonate reaction involving only 2000 pounds per 2,000,000 pounds of soil, seventy impervious clay pots were used. The seven soils used in the pot studies were made alkaline by use of definite amounts of precipitated $CaCO_3$ and exposed, embedded, to the weather for more than a year before the applications of $Ca(OH)_2$. The simultaneous surface applications and mixtures with the upper 3 inches of the soils were sampled after 4- and 10-day periods. Considering the relative meagreness of the treatments, the results appear to justify the conclusion that both surface and intra-soil charges had attained maximum carbonation within 4 days (tables 23 and 24).

17. The carbonation of CaO in soils was further studied in field lysimeters which received only natural rainfall. Applications of 8, 32 and 100 tons were made and mixed throughout the entire 8 inches of soil. Precipitated $CaCO_3$, in chemically equivalent amounts, was used as a check. Both surface soil alone and surface soil underlaid by clay subsoil were considered in the studies (pl. 2).

18. In the lysimeter tanks over 60 per cent of theoretical carbonation occurred in the 8-ton treatments within 17 days, equilibrium having been attained between 218 and 366 days. In the 32-ton tanks, carbonation was gradual up to about 80 per cent of theory, at the end of the second year of exposure, little change having been observed in residual $CaCO_3$ between the end of the second and fourth annual periods. Something over one-half of the 100-ton treatments underwent reversion by the end of the second year of exposure. Little change was definitely recorded beyond that time (tables 25 to 30, and fig. 3).

19. Differential leachings, absorption and other factors recorded during the initial four years are considered in the discussion of the results from the lysimeters (table 31 and fig. 4).

20. The losses of total calcium salts from the several carbonate checks were very constant and independent of the extent of treatment; whereas the losses from the oxide tanks increased with increase in the amount of treatments (table 31 and fig. 4).

21. The leachings from all the oxide and carbonate treatments in the tanks without subsoil were very much richer in lime salts than those of the corresponding tanks which contained subsoil (table 31 and fig. 4).

22. At the end of the first, second, third and fourth years of exposure $CaCO_3$ occurrences were determined in the upper and lower strata, or halves, of the lysimeter soils. Practically equivalent amounts of $CaCO_3$ were found in the upper and lower strata of the 8-ton tanks. In the 32-ton and 100-ton tanks, the $CaCO_3$ contents of the several upper strata were uniformly and decidedly higher than those of the corresponding lower strata for each of the four annual periods, thus showing the influence of the adjacency of the several

upper strata to the movements of aerial atmosphere and also the interception of precipitated CO_2 (tables 32 to 35, and fig. 5).

23. Analyses of the soil atmospheres were made during the third and fourth years after placing. The results indicated a slight and continued depression in the CO_2 content of the 8-ton CaO tanks, suggesting either, or both, partial sterilization or effective alteration of the soil's tendency to condense CO_2 upon its surface (table 36).

24. The subsoil atmosphere withdrawals were much richer in CO_2 than were those obtained from the surface soil (table 36).

25. Tumbler containers were used in an indoor carbonation study of surface and intra-soil applications of $Ca(OH)_2$. Air-dry, moistened and formaldehyde-wetted soils, with and without sand mulches, were included in the experiment (pl. 3).

26. In the tumbler experiments, parallel surface and admixture exposures of 4 and 8 tons $Ca(OH)_2$ per acre surface, showed a much greater carbonation on the surface than within the soil mass, under both air-dry and moist conditions. The sand mulch registered a very decided retardation in the carbonate reaction, in the case of the Volusia silt loam (tables 37 and 38).

27. The comparison observed between the carbonation effected in the moist and in the dry Volusia series showed more extensive final carbonation in the dry series, thus indicating partial sterilization as having been induced in the moistened series. It further appeared that most, if not all, of the CO_2 which effected carbonation of the lime within the air-dry soil was available through infusion of aerial atmosphere into the soil and that the moistened soil experienced less of this aerial infusion (tables 37 and 38).

28. The carbonate reaction, if measured by accumulated $CaCO_3$, was practically inhibited within the Volusia soil treated with 20 per cent formaldehyde (table 39 and fig. 6).

29. In an attempt to maintain sterile conditions during exposure, through the use of 20 per cent formaldehyde, formic acid was produced as were also a sugar and para-formaldehyde, in considerable amounts; thereby were involved side reactions which nullified the apparent indications as to the absence of biological activities (Volusia and Dunkirk) (pl. 4).

30. Plate cultures demonstrated, however, that sterility was attained and maintained by the formaldehyde treatments.

31. In the tumbler experiments with both Dunkirk and Volusia soils the carbonation effected upon the surface was constantly greater than that which was brought about within the soil under air-dry condition and also when moisture was maintained by both water and 20 per cent formaldehyde additions. Again, in the case of the Dunkirk soil, as with the Volusia, the sand mulch effectively retarded carbonation (tables 37 to 42, and figs. 6, 7, 8 and 9).

32. As differing from the Volusia silt loam, the addition of water to the Dunkirk clay loam caused a more rapid and a greater ultimate carbonation

than was attained in the air-dry mixture from both surface applications and soil-mixed treatment. This held for both 4-ton and 8-ton treatments (tables 40 and 41, and figs. 8 and 9).

33. Initial and subsequent analyses of the calcareous check soils showed that the formaldehyde treatment reduced the carbonate initially present as residual from the preliminary $CaCO_3$ treatments (table 43).

34. In supplementary sets of Dunkirk clay loam, under restricted and unrestricted exposures, more than three-fourths of the CO_2 absorbed by the applied lime was derived from the aerial atmosphere, in the case of both 4-ton and 8-ton treatments (table 44 and fig. 10).

35. In simulating the conditions encountered when lime is applied upon the surface of a soil mulch, hydrated lime was exposed at the soil surface in circular containers, some having solid bottoms and some having perforated bottoms, the exposures being made at Knoxville during the month of August. Soil atmosphere diffused upward from the soil and through the perforations to the surface charges of $Ca(OH)_2$ and thus effected a more extensive carbonation than was obtained with the unperforated containers (table 45).

36. Additional exposures with modifications were made at Knoxville during October and November, in containers (as in 35). In this set, diffusions of (a) soil atmosphere and (b) moisture vapor from a saturated asbestos reservoir were permitted to surface-lying charges of $Ca(OH)_2$, as compared to adjacent hydrate which depended in the main upon aerial atmosphere for its supplies of both moisture and CO_2. It appeared that the greater carbonation brought about by upward diffusion of soil atmosphere (35) was in most part due to the moisture diffused and in lesser extent to the CO_2 derived from soil atmosphere diffusion, the greater part of the CO_2 fixation having been derived from aerial atmosphere (table 46).

37. Still further exposures were made at Knoxville during December and January. In this set, intervening trap charges of $Ca(OH)_2$ were placed between the moist soil surface and the overlying surface charges of $Ca(OH)_2$. The surface charges, which were protected by the intervening trap charges, were less extensively carbonated than were the ones devoid of traps and the trap charges showed an increase of CO_2, as a result of the interception of the soil atmosphere CO_2 which diffused to the surface. This again demonstrated that the CO_2 utilized in the carbonation of the surface applications of $Ca(OH)_2$ is in part due to the soil atmosphere but is derived in the main from that furnished by the aerial atmosphere (table 47).

38. Light charges of hydrated lime were mixed with quartz sand in glass cartridges and exposed within the soil at Ithaca during the months of May, June and July. With physical contact thus prevented, the lime charges within the soil underwent a much more rapid reversion to the carbonate than did the same mixtures in similar containers on the soil surface (table 48).

39. The exposures of (38) were repeated at Knoxville during August and September. The increase in CO_2 fixation in the embedded cartridges was

almost twice as great as that found in the cartridges which rested upon the soil surface (table 49).

40. The cartridge container exposures of $Ca(OH)_2$ were repeated during October at Knoxville with a slight modification. Moisture reservoir tubes attached to each end of each cartridge assured an abundance of moisture vapor to the contents of the surface charges, as well as to intra-soil charges. Greater CO_2 absorption again occurred within the soil (table 50 and fig. 11).

41. The surface and soil embedded exposures were again made during the month of December, at Knoxville, considerably larger charges of hydrate having been used. In this case the results were reversed, the soil surface exposures having been more extensively carbonated than were those which were placed within the soil (table 51).

42. Further simultaneous exposures on the surface and within the soil were then made at Knoxville during cool April weather, both 0.6-gm. and 3.0-gm. charges of hydrate having been placed within the cartridges. The surface charges were again more extensively carbonated in each instance than were those placed within the soil (table 52).

43. Still further exposures of 0.6-gm. and 3-gm. charges of hydrate, enclosed within glass cartridges, were made at Knoxville during May and June, after the soil had warmed up. In each instance it was found that more extensive carbonation had occurred where the charges were placed within the soil, the results being in accord with those obtained in making the summer exposures at Ithaca and late summer and fall exposures at Knoxville (table 53).

44. The open sandy types effected a more extensive carbonation than did the heavier types, though the latter were richer in organic matter (table 48).

45. The results reported by Hess, Frear and those of MacIntire from analyses of the Pennsylvania Station plats are considered in their interrelationship. From such a consideration it is pointed out that there has been secured no evidence which would indicate a chemical disintegration of soil organic matter as a result of the use of burnt lime.

46. In an effort to determine whether any of the CO_2 responsible for the formation of $CaCO_3$ was derived from chemical action of the applied $Ca(OH)_2$ upon soil organic matter, soil and heavy charges of $Ca(OH)_2$ were mixed and kept in closed containers for over 4 months under dry and moist conditions, both sterile and non-sterile (table 54).

47. No CO_2 evolutions, as indicative of chemical decomposition of soil organic matter, were obtained from these sealed contacts between 0.4-gm. charges of $Ca(OH)_2$ and 50-gm. charges of each of two soils during a contact period of 126 days, under moist and dry and sterile and non-sterile conditions (table 54).

48. In an effort to intensify the condition of contact between the active solution phase of $Ca(OH)_2$ and soil organic matter, ten soils, some acid and some alkaline, were agitated for periods of from 3 to 15 hours with *saturated* $Ca(OH)_2$, without any indication of chemical or biological decomposition of carbonaceous material (table 55).

49. Six cylinder soils treated with CaO at the rate of 4000 pounds per acre 2,000,000 pounds of soil and six soils treated with an equivalent amount of $Ca(OH)_2$ were compared with six soils which received simultaneous treatments of an equivalent amount of precipitated $CaCO_3$, after the unabsorbed portions of CaO and $Ca(OH)_2$ had become carbonated, in a comparison of the residual organic matter as influenced by the three treatments. No evidence of destruction of organic matter was adduced. A similar result was obtained in a like comparison between four treatments of CaO, four of $Ca(OH)_2$ and four of $CaCO_3$, each chemically equivalent and at the rate of 8000 pounds of CaO per 2,000,000 pounds of soil (tables 56 and 57).

50. In making the organic carbon determinations the wet combustion method of Schollenberger was modified in the absorption technic, by the use of NaOH as an absorbent. The absorption solution was washed into 500-cc. flasks, the Na_2CO_3 precipitated by the addition of $BaCl_2$, and solution made to volume; 200-cc. aliquots of the clear supernatant solution were then titrated for excess of hydrate, with phenolphthalein as an indicator.

51. The experiments as a whole point to certain definite facts which have practical application. Among these are the following:

a. CaO and $Ca(OH)_2$ in 2- to 4-ton applications will revert to the carbonate more rapidly when left on the soil surface than when mixed with a dry mulch or the moist soil.

b. If an oxide or hydrate application is left on the soil surface for several days prior to inworking, the treatment is in effect an application of finely divided $CaCO_3$.

c. Surface applications of oxide and hydrate will, for the most part, revert to the carbonate more rapidly during humid atmospheric conditions.

d. If the oxide or the hydrate is incorporated within the upper zone of the soil, prior to a more thorough dissemination throughout the soil, the concentration thus effected will bring about in the treated zone a temporary and partial sterilization which may have certain beneficial results.

e. The avidity, or affinity, of CaO is for moisture and when this is furnished by the aerial and soil atmospheres and by soil moisture, there is no tendency to effect any *chemical* disintegration of soil organic matter.

f. Neither CaO nor $Ca(OH)_2$ can be considered as chemically destructive of soil organic matter when used in the manner and in the amounts to be considered as practical applications.

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PLATE 1

FIELD CYLINDERS USED FOR EXPOSURE OF BURNT LIME AND HYDRATED LIME (TABLES 11–21)

PLATE .

PLATE 2

FIG. 1. General view of hillside system used for exposure and leaching experiments.
FIG. 2. Inside view of hillside system used for exposure and leaching experiments.

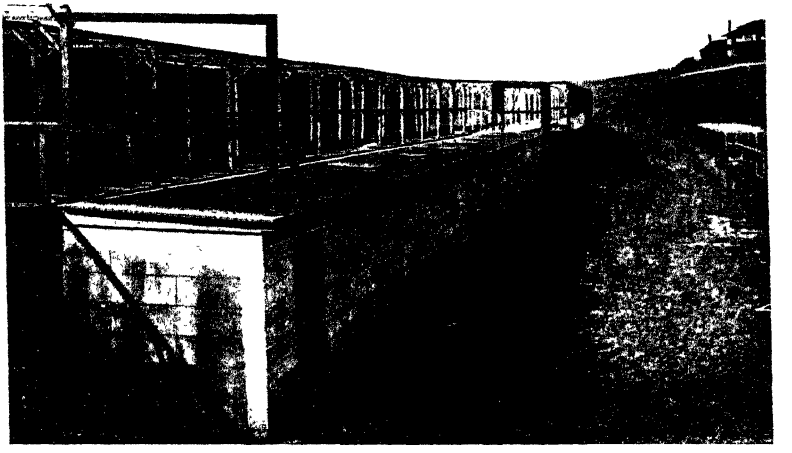


FIG. 1

FIG. 2

PLATE 3

TUMBLER EXPERIMENTS: METHOD OF EXPOSING DUNKIRK CLAY LOAM AND VOLUSIA SILT LOAM SOILS AFTER SURFACE AND MIXED APPLICATION OF $Ca(OH)_2$

PLATE 3

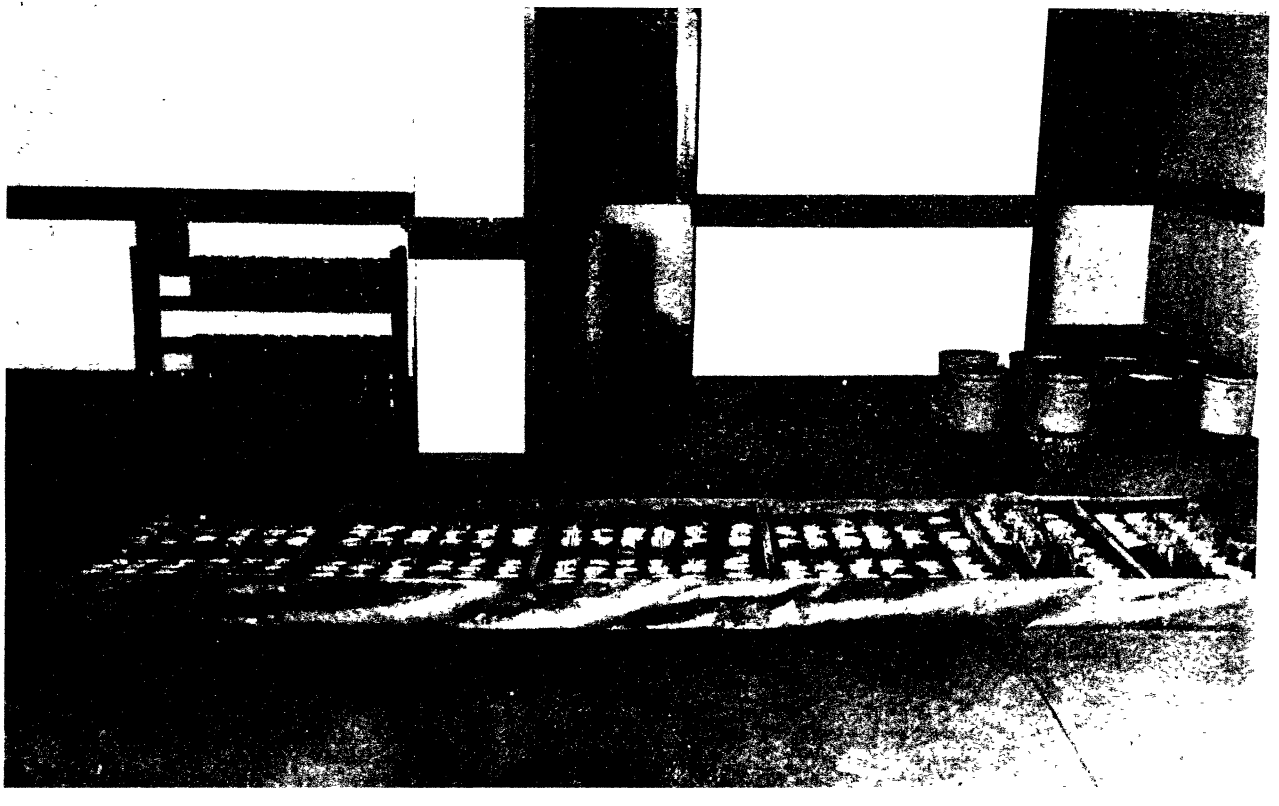
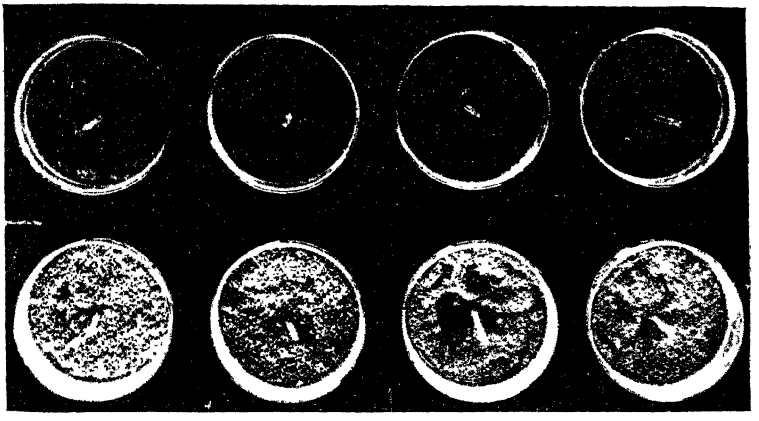


PLATE 4

Showing Surface Accumulation of Para-Formaldehyde Deposited upon the Surface of the Formaldehyde Treated Soils (Lower Row)



EFFECT OF INOCULATION AND LIME ON THE YIELD AND ON THE AMOUNT OF NITROGEN IN SOYBEANS ON ACID SOIL[1]

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In many parts of this state the soil is so low in calcium carbonate that the growth of alfalfa, or even clover, is almost a total failure (2). If the organic matter and nitrogen supply of these acid soils is to be maintained or increased, it is essential that leguminous crops be grown. Fortunately, there are certain legumes which are more tolerant of soil acidity than alfalfa and clover. Chief among these plants which may be of value in Wisconsin are soybeans, vetch, serradella and lupines.

As a soil builder the soybean has few equals. If grown in company with the nitrogen-fixing bacteria of the roots, this plant will absorb large amounts of nitrogen from the air. In other words, soybeans through the efforts of the bacteria, feed heavily upon the air nitrogen instead of upon the soil nitrogen (1). For the farmer on the light soils this property of the soybean is of great value. In the state of Wisconsin it is particularly important that soybeans be given a fair trial since it has been shown repeatedly that this plant will thrive in a soil which is moderately acid.

Several tests have been carried out with these crops on soils of varying reaction. The results of the work with soybeans are reported in this paper. In order to avoid confusion the technical information is given in simplified form.

The experiments reported in this paper were planned to study the influence that additions of limestone with and without inoculation with nodule bacteria exert upon the yield and composition of soybeans. In connection with this work the changes in the nitrogen supply of the soil were studied carefully.

Tests were carried out with soybeans on two important soil types—Plainfield sand and Colby silt loam. The results of both pot and field tests are included in this paper. In one pot test it was possible to make a complete nitrogen balance, i.e., to determine the amount of nitrogen in the soil at the beginning, and at the end, the amount in the crops, in the planted seed, and in the added water.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The sand soils were collected at Sparta and at Hancock, and the silt loam soils were collected at the Marshfield substation and at Curtiss. These soil samples were shipped to the laboratory where they were sieved and potted into 2- and 4-gallon glazed jars. In certain of the experiments, the results of the pot tests have been calculated in terms of pounds per acre. It is difficult to obtain a fair figure, and yet it has been shown repeatedly that pot tests, when properly controlled, give a true index of the nature of certain processes of the soil. For this calculation 1 gm. of plant tissue, or of nitrogen, to each jar of $10\frac{1}{2}$ inches in diameter corresponds to 1 pound to each square rod or 160 pounds to each acre.

The fact that soybeans contain larger amounts of nitrogen than nearly any other plant, renders them especially valuable for forage. Moreover, it is well known that the greater part of this nitrogen is, or should be, obtained from the atmosphere. Thus, the ground on which soybeans are grown, instead of being impoverished, is actually enriched by the nitrogen residue. The experiments upon soybeans which have been carried on for many years at this and other agricultural experiment stations show that soybeans frequently grow well on soils which are moderately acid. In fact, they will often thrive on soils that are too acid to permit the growth of a profitable crop of red clover.

The authors have not found any evidence to support the statement that has been made repeatedly that tubercles in acid soils have little power to gather nitrogen from the air. Undoubtedly, an acid-reacting soil is injurious to the growth of many of the legume bacteria. However, when the bacteria have once gained entrance, and have formed nodules, the soil acidity has little if any effect on the extent to which nitrogen is drawn from the air.

SOYBEANS ON SPARTA SAND

In the spring of 1915 twelve large jars, each containing 19,150 gm. of Sparta sand, were planted to mammoth red clover. Aside from the lime treatments, all of the jars were given a liberal amount of phosphorous and potassium in order that the lack of these elements would not limit crop growth. From these jars three cuttings were made. The benefits from lime and from nitrogen-gathering bacteria on the yield and amount of nitrogen in clover cannot be questioned. Lime at the rate of 3.2 tons per acre, enough to neutralize only half of the soil acidity, increased the growth of red clover more than 50 per cent, as is shown in table 1.

A summary of the results of the clover crop are shown in table 1.

Twenty-six hundredths of a gram of lime to 100 gm. of soil is equal to 3.2 tons per acre, or one-half enough to neutralize all of the active soil acids. Correspondingly, 0.52 gm. is equal to 6.5 tons, or enough to neutralize all of the active soil acids.

TABLE 1

Effect of inoculation and of lime on clover

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	48.55	1604.5	3.31
2	Inoculated	0	53.86	1945.6	3.61
3	Uninoculated	0.26	73.26	2473.8	3.38
4	Inoculated	0.26	76.78	2614.6	3.41
5	Uninoculated	0.52	70.63	2276.9	3.22
6	Inoculated	0.52	77.08	2510.7	3.26

Although red clover has been grown for many years on this soil type it appears that the soil is not thoroughly infected with the nodule organisms. In every case the jars to which the bacteria were added showed a gain in dry matter and in total nitrogen. Possibly the legume bacteria present in these soils are not so active as the bacteria added in the inoculum.

TABLE 2

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS*	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	31.4	1006.0	3.21
2	Inoculated	0	38.9	1469.0	3.78
3	Uninoculated	0.26	52.5	1517.9	2.89
4	Inoculated	0.26	54.7	1898.2	3.47
5	Uninoculated	0.52	44.0	1402.3	3.19
6	Inoculated	0.52	52.6	1920.9	3.65

* Two crops.

In the spring of 1916, without further treatment this Sparta sand was planted to soybeans. The young seedlings were inoculated with a pure culture of bacteria isolated from a soybean nodule. The soybeans made only a fair growth. The plants were cut, and the roots examined for nodules. The inoculated roots showed an abundance of nodules, except in jar 2, the unlimed sand. Here only a few nodules were found. Since the culture used for the inoculation undoubtedly contained millions of active nodule bacteria, it is strange that nodules were not found on the roots of these plants. Perhaps the acid reaction of the soil so injured the bacteria that they were unable to enter the host plant.

The roots of the first soybean crop were returned to the soil and the sand replanted to soybeans. Inoculation was repeated. The second crop made a luxuriant growth. The plants were more than 3 feet tall when harvested.

In every case the inoculated plants were of a deeper green color than the uninoculated plants. The roots were examined for nodules and returned to the soil. All of the plants in the uninoculated soil were free of nodules while the roots in the inoculated soil were thickly studded with nodules. A summary of the results of this study is given in table 2. Unless stated otherwise, the figures represent the average of two or three jars. In many cases two or three crops were grown in each jar.

The inoculated plants showed a gain in dry matter of 7.6 gm., or 24.2 per cent increase as compared with the uninoculated plants. Where lime and inoculation were used together the increase was 23.4 gm. of dry matter, or 74.7 per cent. It is of particular interest to note the effect of varying amounts of lime. Lime sufficient to neutralize one-half of the soil acidity gave the best results.

Besides this gain in dry matter, the inoculated plants contained a higher percentage of nitrogen. Here, as in many other tests, the benefit from the inoculation of soybeans is more noticeable in the gain in nitrogen than in increased crop yield.

SOYBEANS ON HANCOCK SAND

This soil was secured from the Hancock substation. The field from which the soil was secured had been cropped for a number of years without the addition of any fertilizer. Consequently, the soil was in a poor state of fertility. It is not known definitely, but it is improbable that soybeans had ever been grown on this soil. The results of the experiments support this statement. The soil is a light brown sand with little organic matter, and is decidedly acid in reaction. Since this sand is very low in phosphorus and potassium, an application of 0.25 gm. of dibasic potassium phosphate (K_2HPO_4) per kilogram of dry soil was made.

According to the results of acidity tests, this sand would require 2.575 tons of calcium carbonate to neutralize the acid in one acre of 2,500,000 pounds. In the experiments that follow it was arranged to study the effect of different amounts of lime on soybeans. Three amounts of calcium carbonate were used: (a) 0.105 gm. to each 100 gm. of sand (sufficient to neutralize one-half of the active soil acidity), (b) 0.210 gm. to each 100 gm. (sufficient to neutralize all of the active acidity), and (c) 0.315 gm. to each 100 gm. (one and one-half times the amount required to neutralize the active acidity).

The sand was put into 2-gallon jars, 10,940 gm. to each jar, and water added to bring the soil to 60 per cent of saturation. As far as possible the soil moisture was maintained at this point.

On June 24 the jars were planted to Wisconsin Black soybeans. Before planting, the seeds were treated with warm mercuric chloride solution to remove any nodule bacteria. As soon as the young seedlings appeared, the soil was inoculated with a pure culture of the soybean bacteria. The roots

of the plants were examined from time to time by carefully washing away a small amount of sand from one of the plants. Although this is by no means an accurate test, it furnishes information concerning the approximate date of inoculation. Eighteen days afterward, the inoculated plants showed profuse nodule formation, but no apparent difference from the uninoculated in growth. However, one week later the inoculated plants were somewhat deeper green in color than the uninoculated, and slightly larger. The soybeans were cut August 20. The tops were saved for analysis, and the roots

TABLE 3

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	8.9	135.7	1.53
2	Inoculated	0	20.0	639.3	3.20
3	Uninoculated	0.105	14.0	235.4	1.68
4	Inoculated	0.105	21.7	721.5	3.32
5	Uninoculated	0.210	13.3	243.1	1.83
6	Inoculated	0.210	18.1	599.3	3.31
7	Uninoculated	0.315	13.9	256.8	1.85
8	Inoculated	0.315	14.2	448.7	3.16

TABLE 4

Effect of inoculation and of lime on yield and on composition of soybeans

NUMBER	TREATMENT	LIME ADDED PER ACRE	DRY WEIGHT PER ACRE		NITROGEN PER ACRE	
			Total	Increase due to treatment	Total	Increase due to treatment
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Uninoculated	0	2296.2		35.0	
2	Inoculated	0	5160.0	2863.8	164.9	129.9
3	Uninoculated	2625	3612.0	1315.8	60.7	25.7
4	Inoculated	2625	5598.6	3302.4	186.1	151.1
5	Uninoculated	5250	3431.4	1135.2	62.7	27.7
6	Inoculated	5250	4669.8	2373.6	154.6	119.6
7	Uninoculated	7875	3586.2	1290.0	66.3	31.2
8	Inoculated	7875	3663.6	1367.4	115.8	80.8

were examined. The roots from the uninoculated soil were entirely free from nodules while from inoculated soils the roots were thickly studded with large and small nodules. All of the root tissue was returned to the soil.

On March 1 the following spring, the jars were replanted to soybeans. This second crop made only a fair growth. As in the case of the preceding crop, the plant stems and leaves were removed for analysis, and the roots returned to the soil. Before planting the third crop 0.2 gm. of dibasic sodium phosphate (Na_2HPO_4) to each kilogram of soil was added to each jar. On June

2 soybeans were sown and the soil re-inoculated with soybean bacteria. The plants grew better than any of the preceding crops. Three weeks after seeding, the inoculated plants were clearly marked by a deep green color. The uninoculated control remained yellow, while the plants from the limed jars exhibited a green color somewhat lighter in shade than the plants from the inoculated jars. This third and best crop of soybeans was cut July 25, and the tops saved for analysis. On examination of the roots it was found that the uninoculated control jars were free from nodules. The uninoculated and limed soils showed very few if any nodules; whereas the inoculated soils showed an abundance of nodules. The results of this study involved a great number of analyses and considerable data. Obviously, it is preferable to condense the results. Therefore, only the average values are here presented.

Tables 3 and 4 give the results of the first crop yield and nitrogen content of soybeans grown under the four conditions: no inoculation and no lime, inoculation only, lime only, and inoculation plus lime. The lime was applied in three amounts. The figures of table 4 are the same as those of table 3 except that they are calculated to the acre basis. It will be seen from the table that inoculation was far more effective than lime in promoting the growth of soybeans on Hancock sand; although lime alone caused an increase in the crop. It is true that lime and inoculation together gave the maximum yield; however, the difference is not great. The gain in the crop from lime alone is most pronounced in the case of the smallest application. Larger amounts of lime were favorable, but not in proportion.

The determination of the total as well as of the percentage of nitrogen brings out some interesting points. Perhaps the most noteworthy fact is the enormous accumulation of nitrogen in the inoculated plants as compared with that in the uninoculated plants. Here, as in many other experiments, the influence of inoculation on soybeans is noted chiefly in the greater nitrogen accumulation, rather than in any marked gain in the yield of dry matter. The favorable influence of lime on total nitrogen may be accounted for by the increase in soluble nitrogenous compounds of the soil—a process which is well known.

Turning now to the effect of inoculation on the percentage of nitrogen in the crop, the results are most striking. Inoculation alone more than doubled the percentage of nitrogen in soybeans. The application of lime had little effect. Table 5 gives the results of all three crops. The whole evidence points to the importance of having the proper bacteria growing with soybeans if the maximum amount of nitrogen and the largest yields are to be secured. In the absence of bacteria soybeans take large amounts of nitrogen from the soil.

A study has been made of the source and the amount of nitrogen in soybeans with and without nodule bacteria when grown under varying conditions of reaction. For this purpose a careful record has been kept of the amount of nitrogen added to the soil in the seed, and of the nitrogen removed in each

crop. Determinations of the total nitrogen in the soil at the beginning and at the end have been made. The results of these analyses are shown in table 6. These figures represent three successive crops of soybeans on Hancock sand. The results show clearly that nodule bacteria increase the total amount and the percentage of nitrogen in soybeans. In order to emphasize the nitrogen nutrition of soybeans when grown alone and in company with bacteria, the figures of table 6 were used in drawing figure 1. Here the source of nitrogen for soybeans can be seen at a glance.

TABLE 5

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS*	TOTAL NITROGEN IN TOPS*	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	28.5	540.1	1.89
2	Inoculated	0	51.2	1759.6	3.44
3	Uninoculated	0.105	47.5	1292.0	2.70
4	Inoculated	0.105	57.7	1982.2	3.44
5	Uninoculated	0.210	41.8	1099.3	2.63
6	Inoculated	0.210	53.2	1749.6	3.29
7	Uninoculated	0.315	47.7	1213.2	2.54
8	Inoculated	0.315	47.3	1507.8	3.19

* Three crops.

TABLE 6

The source of nitrogen in soybeans

NUMBER		AVERAGE TOTAL NITROGEN PER ACRE			
		Uninoculated	Inoculated	Uninoculated plus lime	Inoculated plus lime
		lbs.	*lbs.*	*lbs.*	*lbs.*
1	Soil at beginning	1165.0	1165.0	1165.0	1165.0
2	Soil at end	1016.5	1034.6	1003.6	1042.3
3	Taken from soil	148.5	130.4	161.4	122.7
4	Crops, leaves and stems	139.3	453.9	333.2	511.7
5	Taken from air	9.2	323.5	171.8	389.0

From this diagram it will be seen that if the portion above ground of a well inoculated legume is removed there is an actual loss in total nitrogen. Although a large part of the nitrogen may be taken from the air, it is wrong to suppose that the crop, i.e., the tops, may be cut, removed, and yet the soil supply of nitrogen be maintained. The evidence obtained from many experiments does not in any way indicate that the roots of legumes excrete appreciable quantities of nitrogenous compounds. In farm practice the result would be different; since the farmer would hardly remove all of the plant tissue, a certain portion would at least be returned to the land either as plant residue or as manure.

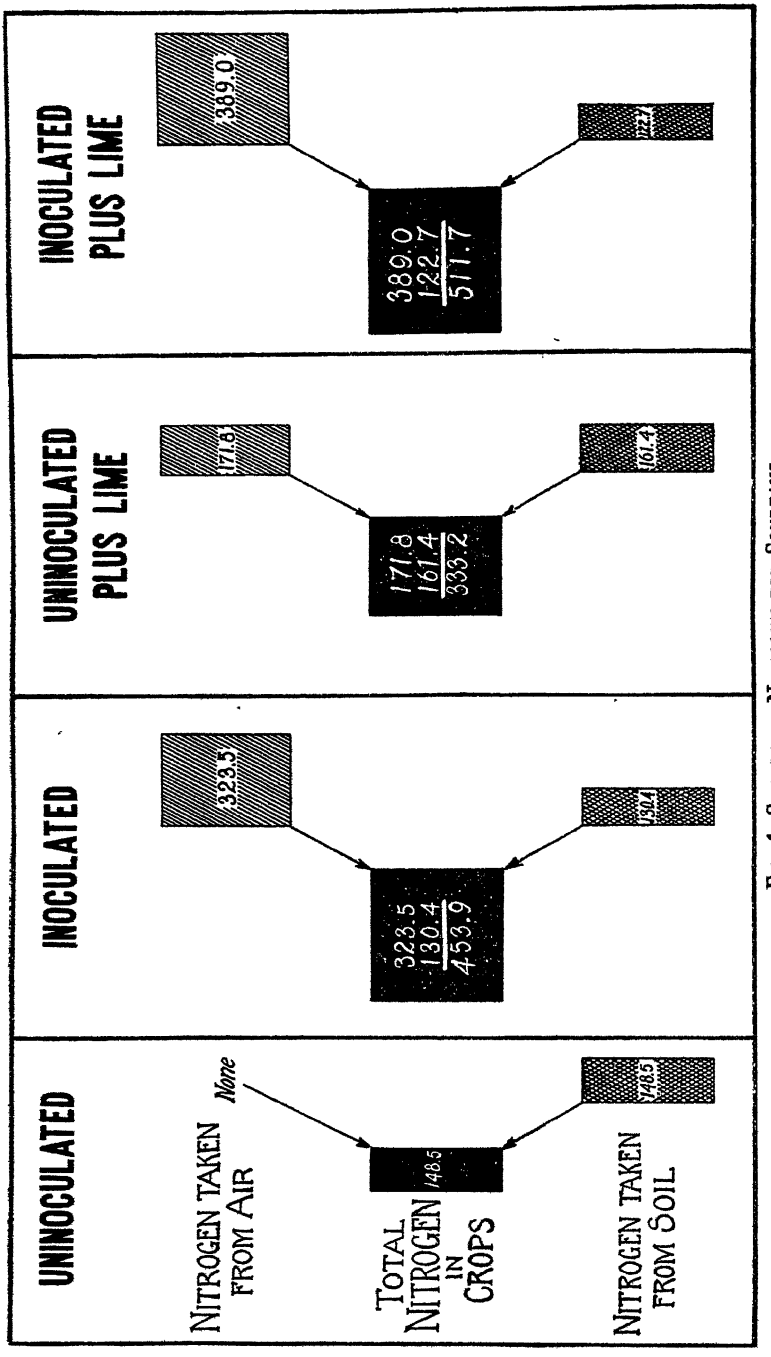

FIG. 1. SOURCE OF NITROGEN FOR SOYBEANS

SOYBEANS ON COLBY SILT LOAM FROM MARSHFIELD

In these tests the same methods were followed as previously outlined. In the spring of 1915 the jars of Colby silt loam soil were planted to red clover. This clover was cut three times and at each cutting the tops were removed for analysis, whereas the roots were allowed to remain in the soil. The yield of the clover in dry matter, and the amount of nitrogen as well as the percentage of nitrogen, are shown in table 7. In general, inoculation failed to

TABLE 7

Effect of inoculation and of lime on red clover

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	56.56	1998.0	3.54
2	Inoculated	0	55.53	2034.7	3.66
3	Uninoculated	0.51	62.90	2279.3	3.62
4	Inoculated	0.51	59.54	2246.7	3.78
5	Uninoculated	1.02	61.58	2317.1	3.76
6	Inoculated	1.02	64.97	2386.4	3.68

TABLE 8

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	65.0	1822.6	2.81
2	Inoculated	0	61.0	2043.9	3.35
3	Uninoculated	0.51	57.3	1597.2	2.79
4	Inoculated	0.51	64.5	2238.2	3.47
5	Uninoculated	1.02	62.0	2007.0	3.24
6	Inoculated	1.02	64.6	2249.9	3.48

increase the crop yield. Apparently Colby soil at Marshfield is well supplied with active clover bacteria. Lime alone caused a slight stimulation in growth of clover. The results show that this soil type may be decidedly acid and yet produce a good crop of red clover.

The following year, March 13, 1916, these jars were planted to Wisconsin Black soybeans. Four days later the inoculated jars of soil were treated with a water suspension of soybean bacteria. The plants made a good growth, but without any apparent difference between the uninoculated and the inoculated jars. On May 10 this first crop of soybeans was cut. The tops were saved for analysis, whereas the roots were examined for nodules and returned to the soil. The roots of the uninoculated plants were free of nodules while the inoculated were thoroughly infected. A second crop was planted May 20,

and the inoculated jars of soil again seeded with soybean bacteria. The plants grew rapidly and a large yield was obtained. Unfortunately, the uninoculated jars of soil became slightly inoculated.

A summary of the results of the nitrogen determinations as well as dry weight of the two crops is shown in table 8. The data of this table deserve careful consideration. Inoculation did not cause any decided change in the yield of soybeans, but it did bring about a marked increase in the nitrogen content of the plant tissue. In every case, the nitrogen-gathering bacteria brought about a gain in the total amount and in the percentage of nitrogen in the crops. Lime and inoculation favored the growth of the soybeans, and to a slight degree, the fixation of nitrogen.

SOYBEANS ON COLBY SILT LOAM FROM CURTISS

For more than forty years this soil at Curtiss had been cropped and no commercial fertilizer or lime returned. To 13,688 gm. per jar of this cropped soil, sodium phosphate and calcium carbonate were added as indicated in table 9. One hundred grams of this soil required 0.848 gm. of limestone to

TABLE 9

Effect of inoculation, of lime, and of phosphorus on soybeans

NUMBER	TREATMENT	ADDED TO 100 GM. OF SOIL		TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		Lime	Na_2HPO_4			
		gm.	*gm.*	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	0	33.28	592.8	1.78
2	Inoculated	0	0	32.01	780.3	2.44
3	Inoculated	0	0.0219	36.86	1063.8	2.89
4	Uninoculated	0.212	0.0219	37.76	674.0	1.78
5	Inoculated	0.212	0.0219	38.19	1138.0	2.98
6	Uninoculated	0.424	0.0219	40.88	805.4	1.97
7	Inoculated	0.424	0.0219	39.58	1284.7	3.25
8	Uninoculated	0.848	0.0219	39.27	1223.2	3.11
9	Inoculated	0.848	0.0219	42.38	1473.4	3.47

neutralize its acidity. The data of this experiment are given in the figures of summary table 9. Examination of the plant roots from uninoculated jars of soil showed the absence of nodules, while roots in inoculated jars of soil were well covered with nodules. It will be seen from the figures of the table that inoculation alone did not increase the crop yield. Apparently, small amounts of lime and phosphorus coupled with inoculation favored plant growth, and gave the most profitable yield.

Turning to the results of the nitrogen analyses, the effect of inoculation is brought out in a very striking way. In every case inoculation increased the amounts of nitrogen in the plant tissue. It is also true that the addition of lime was favorable to an increased nitrogen content of the soybean seed.

SOYBEANS AT THE MARSHFIELD SUBSTATION

A series of plats were planted to Wisconsin Black soybeans as shown in the plan of table 10. No attempt was made to add the limestone on the basis of acid present in the soil. Instead the lime was applied in varying amounts from 1 to 8 tons per acre. Approximately 8.5 tons of lime were required to neutralize all of the soil acids present. The plats were $\frac{1}{60}$ of an acre in size and were arranged so as to have a control plat near one end of the group, and another near the middle of the group. Duplicate plats were used for each test.

When the plants were a few weeks old, tubercles were found in great numbers on the plants from the inoculated soils, while plants from the uninoculated soils showed only an occasional nodule. One of the control plats became

TABLE 10

*Effect of inoculation and of lime on soybeans**

NUMBER	TREATMENT	LIME ADDED PER ACRE	DRY WEIGHT OF TOPS		DRY WEIGHT OF SEEDS	TOTAL NITROGEN OF TOPS		TOTAL NITROGEN OF SEEDS	NITROGEN OF TOPS: SEEDS REMOVED	NITROGEN OF SEEDS
			With seeds	Without seeds		With seeds	Without seeds			
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*†
1	Uninoculated...		3172.0	2795.8	376.2	75.2	45.8	28.4	1.64	7.54
2	Inoculated.....		3294.4	2899.8	394.6	80.5	51.6	28.9	1.78	7.33
3	Uninoculated...	2,000	2756.2	2352.1	404.1	62.3	33.1	29.2	1.42	7.21
4	Inoculated.....	2,000	3450.6	2986.2	464.4	89.1	54.5	34.6	1.86	7.49
5	Uninoculated...	6,000	2693.2	2411.1	282.1	53.2	32.5	20.7	1.36	7.45
6	Inoculated.....	6,000	3702.6	3303.9	398.7	91.2	61.5	29.7	1.86	7.44
7	Uninoculated...	16,000	3424.0	2990.2	433.8	75.8	43.0	32.8	1.45	7.52
8	Inoculated.....	16,000	3955.4	3460.9	494.5	99.9	63.3	36.5	1.83	7.39

* Acre basis.

† Average percentage.

infected to a considerable degree, but it is not surprising that the uninoculated plats became slightly infected when we consider that there were heavy rains, and that the plats were level. The influence of the soil treatment is well illustrated from the results presented in table 10. Inoculation alone, and inoculation with the addition of lime stimulated the growth of soybeans. Far more important than the difference in yield is the increase in nitrogen of the inoculated plants. Here, as in previous experiments, the tops of the plants inoculated with nitrogen-gathering bacteria gave a higher percentage of nitrogen than the tops of the uninoculated plants. The nitrogen-fixing bacteria also increased the dry weight of the seeds, and the total nitrogen content of the seed, but apparently did not increase the percentage of nitrogen in the seeds.

Concerning the effect of lime, it appears that between 1 and 3 tons to each acre gives the most profitable returns. Larger quantities, such as 8 tons to

the acre, failed to cause any marked change in the yield. From these and from other similar experiments it is concluded that soybeans should be inoculated when first seeded, and as a rule, they should be grown a second time on the same land.

SOYBEANS AT CURTISS

In the spring of 1917, twenty plots were arranged on cropped Colby silt loam and on May 10 planted to Wisconsin Black soybeans. The plots were $\frac{1}{186}$ acre in size. The summer was very dry, and an early frost damaged the crop severely. It was harvested September 1, and samples were drawn for analysis. The treatments of the different plots, together with the crop yields and nitrogen content, are recorded in table 11. Here as in many other field and pot experiments there is but little increase in the crop production on the addition of nitrogen-fixing bacteria to such soils.

TABLE 11

*Effect of inoculation and of lime on soybeans**

NUMBER	TREATMENT	BONE MEAL ADDED PER ACRE	LIME ADDED PER ACRE	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*per cent*
1	Uninoculated	400		2622.9	83.2	3.17
2	Inoculated	400		2425.1	84.7	3.51
3	Uninoculated	400		2496.0	83.0	3.33
4	Inoculated	400		2452.2	88.0	3.60
5	Uninoculated	400	2,000	2417.5	81.0	3.36
6	Inoculated	400	2,000	2749.7	95.8	3.53
7	Uninoculated	400	6,000	2796.5	96.3	3.43
8	Inoculated	400	6,000	2875.0	110.9	3.87
9	Uninoculated	400	10,000	2736.1	94.7	3.46
10	Inoculated	400	10,000	2943.0	107.8	3.69

* Acre basis.

SUMMARY

It is noteworthy that in every test the percentage of protein in the tops was higher in the case of the nodule-bearing plants than with the plants free of nodules. Although the absence of the nodule bacteria renders it impossible for the soybean plant to utilize the nitrogen of the air, the effect is not always apparent. In parts of Wisconsin, soybeans will produce large crops in the absence of the nodule bacteria, but in such cases, the entire nitrogen supply of the plant is taken from the soil.

Inoculation of soybeans grown on Sparta sand increased the yield of dry matter. The yield was further increased when inoculation was supplemented by an application of lime. Sufficient lime to neutralize one-half of the active soil acidity was sufficient to produce maximum yields. The percentage of nitrogen in the crop and the total amount was increased by inoculation.

The crops grown on the sand from Hancock yielded better when inoculated than when uninoculated. In fact, inoculation was more effective in increasing the yield than was lime. Lime in small amounts and inoculation together produced the best yields.

The nitrogen content of those crops which were inoculated was considerably higher than that of the uninoculated crops. When the crops are not inoculated all of the nitrogen is drawn from the soil, but when the proper bacteria are present a large proportion of the protein nitrogen is drawn from the air.

When the entire crop is removed from the soil, leaving only the roots and stubble, there will be less nitrogen in the soil than there was before the crop was grown, but if the crop is turned under as a green manure, if properly inoculated, it will add large quantities of nitrogen to the soil.

Inoculation was not as effective on the Colby silt loam as on the sandy soils in increasing yields, but influenced the percentage of nitrogen, and consequently the total amount in the crop.

Soybeans if properly inoculated will utilize nitrogen from the air and thus enrich the soil, provided the crop residue is returned to the soil. It is not necessary to plow the crop under if the crop is fed on the farm. The manure can be returned to the soil, and in this way be utilized in the improvement of the land. Small additions of limestone to acid soils render the inoculated soybean plant more active in absorbing air nitrogen.

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SOIL ACIDITY: II. ITS RELATION TO THE ACIDITY OF THE PLANT JUICE[1]

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In the first paper (7) of this series the general relation of soil acidity to plant growth was discussed. It was stated, with certain reservations, that the main specific harmful influence of soil acidity on certain plants is that it prevents these plants from securing at a sufficiently rapid rate the calcium as the carbonate or bi-carbonate which is needed to neutralize and precipitate certain acids in the plants themselves, which are probably largely by-products produced as the result of certain vital reactions in the growth of plants. If calcium in these forms is not furnished at a sufficiently rapid rate, then the rate of these reactions is lowered accordingly, as is also the rate of plant growth. If this is the case, then it seems that the juice of at least some plants growing on acid soils should be slightly more acid than that of plants growing on non-acid, or limed acid soils. That this condition usually holds was briefly stated in the first paper. The data previously referred to relating to this phase of the subject are given in this paper.

Except where otherwise stated, the plants were grown in 2-gallon pots in the greenhouse on limed and unlimed medium, to strongly acid, soils. In the limed series, crushed limestone was added, according to the acidity, in amounts equivalent to common field applications. The determinations on the corn plants were made when the plants were about 15 inches high. The other plants were used shortly before blooming.

With three exceptions, later given, the juice was secured as follows: The plants were cut within several inches of the ground and thoroughly macerated by passing them through a tinned meat grinder. The juice was then expressed with a press. In most cases before making the determination the juice was centrifuged about 5 minutes to remove coarse material in suspension. This was found more satisfactory than filtration. The determinations were then made immediately on this juice without the addition of water.

In three cases of alfalfa—(a), (b) and (c), table 1, the juice was obtained in a different manner from that just explained. In the cases of (a) and (b) 10 gm. of the plant tops were thoroughly macerated and then worked up with 100 cc. of water and filtered. The filtered extract was then used. In

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the case of (c), 15 gm. of the tops were macerated with 10 gm. of quartz, and the juice pressed out. Of this juice 5 cc. were diluted with 50 cc. of water, and the determination made on this. These three determinations were made in 1916 with the assistance of N. E. Loomis.

The determinations of the acidity,[2] or hydrogen-ion concentrations, were made electrometrically at 25°C. with an apparatus slightly modified from that described by Loomis (4) and Acree. The results, or acidity, are expressed in terms of the P_H symbol of Sorensen. Corrections were made for pressure and variations of the calomel electrode as suggested by Loomis (5) and Acree.

TABLE 1

The acidity in terms of hydrogen-ion concentration of the juice of plants (tops) grown on unlimed and limed acid soils

KIND OF PLANT	P_H VALUE OF THE JUICE	
	Unlimed acid soil	Limed acid soil
Alfalfa, *Medicago sativa* (a)	5.78	6.03
Alfalfa, *Medicago sativa* (b)	5.79	5.88
Alfalfa, *Medicago sativa* (c)	6.28	6.46
Alfalfa, *Medicago sativa* (d)	5.61	5.67
Alfalfa, *Medicago sativa* (e)	5.80	5.79
Alfalfa, *Medicago sativa* (f)	5.62	5.57
Clover, *Trifolium pratense* (a)	5.69	5.83
Clover, *Trifolium pratense* (b)	5.81	5.90
Clover, *Trifolium pratense* (c)	5.69	5.74
Clover, *Trifolium pratense* (d)	5.73	5.80
Lupine blue, *Lupinus hirsitus* (a)	5.54	5.45
Lupine blue, *Lupinus hirsitus* (b)	5.65	5.45
Soybean, *Glycine hispida*	5.81	5.94
Buckwheat, *Fagopyrum esculentum*	4.02	4.05
Corn, *Zea mays*	5.21	5.31
Rape, *Brassica napus*	5.10	5.18

In table 1 the results of the limed and unlimed series are given. In 12 cases out of 16 the plant juices are more acid on the unlimed series. Only in 2 cases out of 6 with alfalfa and in the case of lupine are the results reversed. The blue lupine is sometimes injured by liming; and hence an important question is raised whether or not certain injuries result in an increase in acidity. In the two reversed cases of alfalfa (e), and (f), the differences are quite small and it is possible that the reversed results may be due to small differences which existed in moisture supply, time of day plants were cut, and way in which juice was handled, although some attempt was made to make these conditions uniform. It is to be noted that the acidity of both the limed and unlimed alfalfa in these cases is quite high for alfalfa, and that evidently even in the limed series the plants were not getting sufficient lime.

[2] In this paper acidity and hydrogen-ion concentration are used synonymously.

The results given in table 2 emphasize this point. The alfalfa from which the results of table 2 were obtained was grown out-of-doors on a rich neutral soil, and the acidity of the plant juice is much less than in the two reversed cases.

The data in table 2 were obtained in studying the effect of various conditions of determination indicated in the table, on the acidity of the plant juice. Number 2 is considerably more acid than number 1, indicating that the expressed juice becomes more acid on standing. The values obtained in numbers 1 and 3 while the weather was warm and dry indicate a slightly higher acidity than the comparable values in numbers 4 and 7 obtained after a heavy rain.

TABLE 2

Effect of various conditions of determination on the acidity in terms of hydrogen-ion concentration of alfalfa (tops) juice

Number	CONDITIONS OF DETERMINATION					P_H VALUES
	Date	Weather	Time of day when cut	Time when juice was expressed	Time when determination was made	
1	May 18	Clear, warm, dry	6.00 a.m.	8.00 a.m.	8.30 a.m.	5.97
2	May 18	Clear, warm, dry	6.00 a.m.	8.00 a.m.	5.12 p.m.	5.86
3	May 18	Clear, warm, dry	2.00 p.m.	2.30 p.m.	3.45 p.m.	6.00
4	May 19	Heavy rain previous night, warm	8.00 a.m.	8.30 a.m.	9.45 a.m.	6.04
5	May 19	Heavy rain previous night, warm	8.00 a.m.	10.30 a.m.	10.59 a.m.	6.05
6	May 19	Heavy rain previous night, warm	8.00 a.m.	1.00 p.m.	1.40 p.m.	6.18
7	May 19	Heavy rain previous night, warm	2.30 p.m.	2.45 p.m.	2.57 p.m.	6.11
8	May 19	Heavy rain previous night, warm	2.30 p.m.	3.30 p.m.	4.00 p.m.	6.10

A comparison of number 1 with number 3, and number 4 with number 7, shows that juice from plants cut in the morning is more acid than that from plants cut later in the day, indicating an accumulation of acids at night which were partly used up in the day time, due to diurnal changes in plant processes. A comparison of numbers 4, 5, and 6 indicates that when plants are cut and kept several hours before expressing the juice and making the determination, the acidity may become slightly less.

These data in table 2 emphasize the importance of standardizing the conditions of making the determinations, especially when small differences are to be measured and used in a comparative way. In studying the effect of one factor, like liming, on the acidity of plant juice, other factors like moisture supply, time of day material is cut, and methods of handling the juice, must be made uniform. It appears that after a plant is cut, and especially

after the juice is expressed, the determination should be made as quickly as possible.

Another factor in this connection is the question whether or not the roots and tops of the same plant are of the same acidity Three comparative sets of determinations were made, and are given in table 3. In two cases the roots are slightly more acid than the tops and in the third case the tops are more acid. In this latter case, which is with alfalfa, the plants were grown in pots on a strongly acid soil, and the acidity of both the tops and roots is high for alfalfa, since an acidity of about P_H. 6.0 is normal for alfalfa, as indicated by the other set of figures, and also those in table 2 in which cases the alfalfa was grown out-of-doors on a neutral soil.

TABLE 3

Acidity in terms of hydrogen-ion concentration of the juice of tops and roots

KIND OF PLANT	KIND OF SOIL	P_H VALUES OF THE JUICE	
		Tops	Roots
Clover	Acid	5.74	5.71
Alfalfa	Neutral	6.00	5.84
Alfalfa	Acid	5.61	5.72

The clover was also grown in pots on an acid soil but the acidity found is not far from normal for clover. Apparently the roots are often more acid than the tops, although the reverse may also be the case. More determinations are needed to show how the different parts of the same plant vary in acidity. The results of Kappen (3) show a higher acidity in the tops than in the roots, both as regards actual and total acidity.

The importance of a proper regulation of the acidity of plant juices or protoplasm has been indicated by Kappen (3) who states that the plant juice acidity of each species of plant is specifically adapted to the physiological processes of that plant. The effect of acidity on plant enzyme action is emphasized by him.

The importance of a proper actual plant acidity on oxidase activity has been pointed out by Reed (6) and Bunzell (1). Some unusually high acidities of plant tissues or protoplasm have been reported by Haas (2), who worked largely with fruits. These results indicate that some life processes go on normally in the presence of a high acidity.

Kappen made acidity determinations by means of the gas chain, and also by titration of a considerable number of plant roots and some tops. It is in the green tops and in the leaves especially that the most active life processes take place; and hence the determinations reported in this paper were made almost entirely on the tops. Kappen made one set of comparative determinations on limed and unlimed plant roots, and that was with the yellow lupine. This was made to study the lime sensitiveness of the lupine.

He found the limed plant roots less acid than the unlimed. This is opposite to what the writers found in the tops of the blue lupine.

A relation between plant juice acidity and disease conditions and immunity has been indicated by Wagner (8). Soil acidity, by affecting plant juice acidity, may have a relation to certain plant diseases. This phase of the subject deserves further investigation.

SUMMARY

There are considerable differences in the acidities of the juices of different species of plants. The acidity of each species of plant, while it may vary to an extent easily measurable, is however, usually limited to a rather narrow range. Undoubtedly for each species of plant there is a certain acidity which is most favorable for the life processes of that species. Unquestionably in many cases soil acidity, by limiting the supply of lime available for plants, affects the acidity of the juice or protoplasm of these plants. The importance of a proper regulation of plant acidity in relation to vital plant processes should be noted.

In the life processes of plants, acids are formed, some of which are probably simple by-products. Lime and other bases are needed to neutralize these acids. Plants high in protein are usually high in lime and other bases indicating the formation of acids in protein synthesis. If the supply of lime and other bases is inadequate, the acidity of the plant juice rises to a certain extent which is limited because the accumulation of acids probably decreases the rate of the processes which produce acids. A condition of self regulation thus probably exists and death due to over-acidity is prevented. Slow growth and a weakened condition, however, result as is the case with plants of high lime requirement growing on acid soils.

In plants there are many "buffer" substances which to a certain extent help to maintain a more uniform acidity and prevent rapid and exeessive alterations, as would result especially from diurnal changes in plant processes. Bases are however usually needed in the formation of these "buffer" substances.

There are many conditions which affect the acidity of the plant juice. Some of these conditions are indicated in this paper. Before final conclusions can be made regarding this subject, much more investigation is needed. The proper method of carrying on this investigation, and especially the technique of making the acidity determinations, needs to be standardized. An effort to do this is being made in this laboratory.

The writers are indebted to Dr. S. F. Acree for valuable suggestions and the use of his gas chain apparatus.

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THE OXIDATION OF VANILLIN TO VANILLIC ACID BY CERTAIN SOIL BACTERIA

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The association of aldehydes with infertility in soils, the isolation of aldehydes from the soil and their harmful effects on plant growth, both in water culture and in the soil, are subjects which in the past few years have engaged the attention of a number of workers, notably Schreiner, Reed, Shorey, Skinner, Sullivan, Noll, Davidson, Fraps, Upson and Powell, and Funchess.[1]

Of particular interest to the question of the relation of aldehydes to soil infertility was the discovery that the aldehyde vanillin persists in some soils but does not persist in others. This appears to be due to the fact that the destruction of vanillin in the soil is chiefly a bacterial process (11). In some soils vanillin-destroying bacteria are present and the soil conditions are favorable for their action on vanillin, while in other soils either the suitable bacteria are absent or conditions are unfavorable for their destructive action on vanillin. A bacterium which uses vanillin with facility as an energy and carbon source, was isolated by one of us from Alabama soil (11).

In the present paper studies are reported which show that in solution cultures consisting of pure inorganic salts and vanillin as the only source of carbon, vanillin is oxidized by this bacterium to vanillic acid and the vanillic acid formed is destroyed. By the use of colorimetric methods the formation of vanillic acid from vanillin and the destruction of the vanillic acid so formed has also been studied.

THE IDENTIFICATION OF VANILLIC ACID AS A PRODUCT OF THE DECOMPOSITION OF VANILLIN BY A SOIL BACTERIUM

Vanillic acid was identified as a product of the decomposition of vanillin by soil bacteria by extracting it with ether from a culture solution containing vanillin, in which a pure culture of vanillin-destroying bacteria had grown.

[1] For a summary and discussion of this work see Reference no. 12.

In a 2-liter flask, 250 cc. of a mineral nutrient solution[2] containing vanillin were placed. The flask and contents were sterilized at 15 pounds pressure for 15 minutes and inoculated with a pure culture of the bacterium referred to above. The flask was incubated at 25–30°C., until the testing of a small portion of its contents by the acid nitrate of mercury reagent described by Estes (2) showed the presence of little or no vanillin while the phenol reagent described by Folin and Denis (3, 4) gave a strong reaction. This usually required from 4 to 5 days' incubation. An ether extract of the contents of the flask at this time yielded a whitish crystalline material consisting almost entirely of vanillic acid.

The identity of this decomposition product with vanillic acid was demonstrated by:

(1) The determination of its melting point and mixed melting point with pure vanillic acid.

(2) Comparison of crystalline form and solubilities with those of vanillic acid.

(3) Color reactions.

(4) Neutralization equivalent.

(5) Methoxyl determination.

(6) Micro-combustion.

The crystalline material obtained by ether extraction as described above was obviously impure, being of a dirty grey color and having the odor of vanillin. The material was dissolved in hot water and filtered. To the filtrate lead acetate solution was added, which in no case caused a precipitate. Ammonia was then added to the solution and the heavy white precipitate which formed was filtered and well washed with distilled water. The precipitate while still moist was ground in a mortar with a little water, suspended in hot water and decomposed with hydrogen sulfide. (It was found that the complete removal of lead from the compound was at times difficult and that the conditions best suited for the removal of lead were large volumes of water and precipitation while hot. In some cases it was found necessary to treat more than once with hydrogen sulfide.) The solution was boiled to expel the excess of hydrogen sulfide, filtered and concentrated on the water bath until crystallization began. The solution was brought to boiling to dissolve all of the crystals which had formed, and the dish containing the hot solution was then chilled and the solution stirred vigorously

[2] The composition of this solution was:

$NaNO_3$	0.1 gm.
K_2HPO_4	0.1 gm.
$MgSO_4$	0.05 gm.
NH_4Cl	0.1 gm.
KCl	0.05 gm.
Vanillin	0.1 gm.
H_2O distilled	200.0 cc.

in order to cause rapid crystallization. The crystals which formed were filtered by suction, taken up in hot water, decolorized with a little pure carbon black, filtered and recrystallized as before. The crystals obtained were masses of long white silky needles which gathered together in clusters.

These crystals were identical in appearance with crystals of vanillic acid purified by passing through the lead salt and crystallized in the manner described.

When the vanillin decomposition product was heated it had a strong odor of vanillin, which is characteristic of vanillic acid. When the decomposition product was heated above its melting point it sublimed in long prisms which were identical in crystalline form with the crystals obtained on subliming pure vanillic acid.

The vanillin decomposition product and pure vanillic acid gave the same color reactions. No color was produced on the addition of a dilute solution of ferric chloride to the dilute solutions tested; on treating a dilute solution of each with a dilute solution of acid mercuric nitrate and heating, a faint pink color was produced in each, and with further application of heat this color faded and a grey precipitate was formed; on treating the dilute solutions with Folin's phosphotungstic phosphomolybdic reagent and then adding sodium carbonate solution in excess, the same shade of blue color developed.

A study of the melting points of the vanillin decomposition product and of vanillic acid showed that the former melted at 210.5–211° (corr.). Vanillic acid melted at 210.5–211°(corr.) and a mixture of the two preparations melted at 210.5°. Tiemann (13) who first prepared vanillic acid found that it melted at 211–212° (uncorr.) and Tiemann and Reimer (16) found that vanillic acid purified by passing through the lead salt melted at 211–212° (uncorr.). Later Tiemann (15) states that the melting point is 207° and not 211° as previously reported. Perkin and Martin (9) found the melting point of vanillic acid to be 205–206°. The melting points given by the text books on organic chemistry are apparently taken from the later determinations.

In taking our melting points we used an apparatus provided with a stirring device similar to that described by Menge (7) . We used 6-inch Anschutz thermometers standardized by the U. S. Bureau of Standards, no stem correction being necessary. Both the crystals of the decomposition product and those of pure vanillic acid melted with slight coloration.

A study of the neutralization equivalent of the vanillin decomposition product and of pure vanillic acid was made. Ostwald (8) has studied the dissociation constant for vanillic acid and gives $K = 0.00298$. The indicator to be used in this case was found to be methyl red, titrating to the full yellow color, although both brom-cresol-purple and phenolphthalein were also tried. The neutralization equivalent determined by the use of indicators was:

	PHENOL-PHTHALEIN	BROM-CRESOL PURPLE	METHYL RED
Vanillic acid	151.2	163.9	168.7
		160.7	
Vanillin decomposition product		161.3	171.4

The theory for the neutralization equivalent of vanillic acid, $C_8H_8O_4$ is 168. All of the titrations were made with the use of direct color comparisons.

A determination of the methoxyl group in both the vanillin decomposition product and in pure vanillic acid was made by the method of Zeisel (18), the apparatus described by Benedikt and Grüssner (1) being used. The results were as follows:

COMPOUND	METHOXYL FOUND	METHOXYL BY THEORY
	per cent	*per cent*
Vanillic acid	18.76	18.46
Vanillin decomposition product	20.16	

Combustions were made on the vanillin decomposition product and pure vanillic acid by the micro-combustion method developed by Wise[3] (17). An examination of the vanillin decomposition product used for combustion showed that it contained a very small amount of lead. The lead was determined by oxidizing the organic matter with a mixture of sulfuric and nitric acids and weighing the lead as lead sulfate. The results of the combustion of the vanillin decomposition product are given below as found and as corrected for the lead present. No lead could be found in the purified vanillic acid used.

COMPOUND	CARBON	HYDROGEN
	per cent	*per cent*
Pure vanillic acid	56.15	4.88
	56.65	4.67
Vanillin decomposition product	55.20	4.70
Vanillin decomposition product corrected	56.66	4.83
Theory for $C_8H_8O_4$	57.12	4.79

From the analytical data given above the vanillin decomposition product was identified as vanillic acid.

[3] The authors desire to express their thanks to Dr. Wise for his kindness in making these combustions for them.

THE FORMATION AND DESTRUCTION OF VANILLIC ACID

In solution cultures containing nutrient mineral salts and vanillin and inoculated with the bacterium referred to above the vanillin is oxidized to vanillic acid, which accumulates in considerable amount in the solution and is then destroyed. These facts were developed in attempts to explain the results which were obtained in using the phenol reagent to determine the vanillin content of solution cultures which had been inoculated with the vanillin-destroying bacterium. When the vanillin content of mineral nutrient solution cultures which had been inoculated with the vanillin-destroying bacterium was determined by the phenol reagent it was found that the vanillin content apparently rose enormously in the early stages of digestion but later decreased to zero. When the Estes reagent was used for making the determination the vanillin content showed no such apparent increase but uniformly decreased to the zero point.

The details of an experiment[4] will illustrate these statements.

A number of cultures were prepared containing 40 cc. of mineral nutrient solution[5] in a 150-cc. Erlenmeyer flask. The reaction of the solution was made −2, Fuller's scale, with NaOH. The flasks were sterilized at 15 pounds pressure for 15 minutes and after sterilization 10 cc. of a sterile solution of commercial vanillin were added to each flask. All flasks, save some checks, were inoculated with three drops of a suspension of a pure culture of the vanillin-destroying bacterium. Two cultures and a check flask were analyzed daily with both the acid nitrate of mercury and the phenol reagent.

In making the color determinations with the Estes reagent 1 cc. of the reagent was added to 5 cc. of the culture solution. The mixture was diluted with water and boiled for 5 minutes on a hot plate. The solution was then filtered and made up to 50 cc. with distilled water. The color developed in a similar way by 5 cc. of a solution of 200 parts per million of vanillin was used as a standard. The phenol reagent was used as described by Folin and Denis with the clarification by means of lead acetate omitted. No considerable degree of accuracy is claimed for these determinations, as no attempt was made to vary the concentration of the standard with the variation in the apparent amount of vanillin present in the cultures. In the temporary absence of a colorimeter the comparisons were made with colorimeter tubes of equal diameter.

The results are presented in table 1 and figure 1. In the first column of the table the time in days from the day of inoculation is given. The

[4] The assistance of A. B. Massey in performing this experiment is acknowledged.

[5] The mineral composition of this solution was:

K_2SO_4	0.037 gm.
$NaNO_3$	0.100 gm.
$CaH_4(PO_4)_2.H_2O$	0.071 gm.
Water (distilled)	3000 cc.

figures in the second column are the vanillin contents in parts per million as determined by the Estes reagent. Column 3 represents the values obtained with the phenol reagent expressed in parts per million of vanillin. In the figure the time in days is given on the abscissa and the vanillin content of the cultures in parts per million as determined by the Estes reagent and by the phenol reagent on the ordinate.

From the data in table 1 and the curves in figure 1 it can be noted that the vanillin as determined by the Estes reagent uniformly decreased, sharply at first, more slowly later, until no vanillin was found on the eighth day from the day of inoculation. Using the phenol reagent to determine the vanillin, however, the vanillin content rapidly increased until on the third

TABLE 1

Data showing formation and destruction of vanillic acid

TIME FROM INOCULATION	VANILLIN BY ACID NITRATE OF MERCURY	VALUES WITH PHENOL REAGENT EXPRESSED AS P.P.M. OF VANILLIN
	p.p.m.	*p.p.m.*
Average of checks	249.0	285.0
1 day	257.0	282.0
2 days	177.0	343.0
3 days	56.0	504.0
4 days	23.0	482.0
5 days	22.0	270.0
6 days	26.0	151.0
7 days	10.0	85.0
8 days	0.0	80.0
9 days		17.0

day apparently almost twice as much vanillin was present as was originally added to the solutions. From that point the vanillin decreased until by the ninth day it was practically exhausted.

An examination of the color produced by vanillin and vanillic acid with Estes' reagent and by vanillin and vanillic acid with the phenol reagent explained the anomalous results secured with the phenol reagent.

Pure vanillic acid was prepared by the method already described and pure vanillin was prepared by the following method:

Commercial vanillin was dissolved in ether and the ether extract was shaken out with a freshly prepared sodium bisulfite solution. The sodium bisulfite solution was then acidified with dilute sulfuric acid and as much as possible of the sulfur dioxide was removed by violent shaking of the flask. The acidified solution was then shaken out with ether which had been previously purified from other aldehydes by shaking with a little saturated sodium bisulfite solution. The ether solution was concentrated until all of the ether had evaporated and the molten mass of vanillin was dissolved in boiling water. The solution was filtered while hot and the vanillin crystallized by placing

the beaker in chipped ice. The crystals were filtered off in a Buchner funnel and recrystallized twice more. The pure vanillin was then dried on a porous plate in a desiccator over calcium chloride. It was found to melt at 80.5–81° (corr.).

Experiments with Estes' reagent. When a solution of vanillin was heated with Estes' reagent a clear pink-colored solution resulted. Vanillic acid, however, first produced a pink color which on further heating faded and in

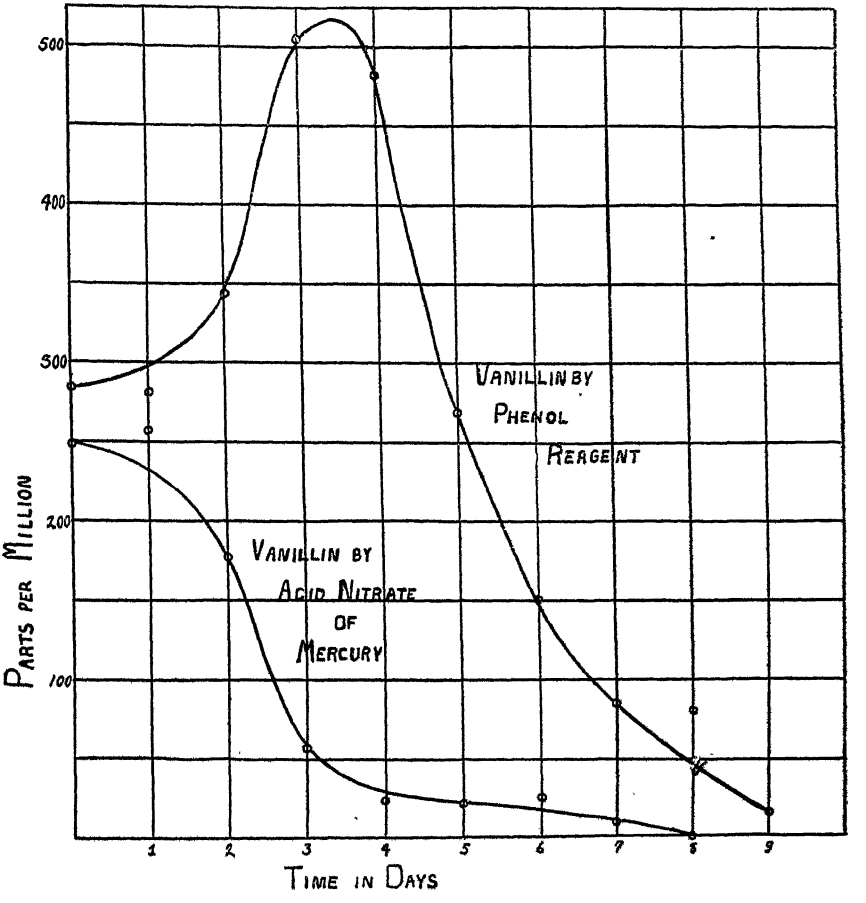

FIG. 1. DIAGRAM SHOWING FORMATION AND DESTRUCTION OF VANILLIC ACID

its place an easily filtered gray or black precipitate was formed. The amount of pink color remaining in the vanillic acid solution depended on the amount of heating. The color produced by heating 10 cc. of 100 parts per million of vanillic acid and 1.5 cc. of Estes' reagent on the steam bath for 20 minutes was about 3 per cent of that produced by the same concentration of vanillin. When the solutions were boiled for five minutes on a hot plate and allowed

to cool the color produced by the vanillic acid was too weak to be read by the Schreiner colorimeter.

Experiments with Folin's reagent. With Folin's reagent both vanillin and vanillic acid were found to produce a deep blue color, the color produced by the vanillic acid being of a slightly different shade of blue and considerably deeper. Thus 5 cc. of 200 parts per million of vanillin were treated with 5 cc. of the phenol reagent, allowed to stand 10 minutes, and made up to 50 cc. with saturated sodium carbonate solution. After standing 15 minutes the solution was filtered and made up to 100 cc. with distilled water. Comparison in a Schreiner colorimeter of the color produced with that developed in a solution of vanillic acid of 200 parts per million treated in the same way showed that the color produced by the vanillic acid was about 1.8 times as strong as that developed by the vanillin. Using solutions of 100 parts per million, a factor of about 1.5 was obtained.

Determinations of vanillin and vanillic acid in mixtures. Since vanillin and vanillic acid both produced a blue color with the phenol reagent and vanillin alone reacts with the Estes reagent to any considerable extent it was thought that a method could be evolved for determining vanillin and vanillic acid in mixtures by using the two reagents. The method proposed consisted in making a determination colorimetrically of the solution containing vanillin and vanillic acid, first with the acid nitrate of mercury reagent described by Estes, using a suitable solution of vanillin as a standard; and, second, with the phenol reagent described by Folin and Denis, using a suitable solution of vanillin as a standard. The results with the Estes reagent should give the quantity of vanillin present. The difference between the results obtained with the Folin's reagent and those obtained with the Estes reagent divided by a suitable factor should equal the vanillic acid.

It was found that by using a standard of 100 parts per million vanillin could be determined by the Estes method in concentrations of 25 to 400 parts per million in the presence of from 50 to 800 parts per million of vanillic acid. On the other hand, vanillic acid could not be accurately estimated within such a wide range of concentration. With a strong concentration of vanillin, 250 to 400 parts per million, and a weak concentration of vanillic acid, 25 to 50 parts per million, the results with the phenol reagent were far too low. With other mixtures the factor necessary was found to vary from about 0.7 to about 2.0. Thus with a standard of 200 parts per million of vanillin a mixture containing 175 parts per million of vanillin and 100 parts per million of vanillic acid gave a result of 296 parts per million with the phenol reagent. Since the mixture contained 175 parts per million of vanillin, 121 parts per million of the result were due to the vanillic acid. Dividing this by the actual amount of vanillic acid present, the factor of 1.21 was obtained. In a mixture of 25 parts per million of vanillin and 200 parts per million of vanillic acid the factor was 1.91. With a standard of 100 parts per million the factor found for a mixture of 300 parts per million of vanillin and 100 parts per million of vanillic acid was 0.76.

The results, therefore, indicated that without a further and more complete study vanillic acid could not be accurately determined quantitatively by the method proposed in mixtures of vanillin and vanillic acid.

Sufficient information was secured, however, to permit an interpretation of the results obtained in the bacteriological experiment mentioned above. The results with the Estes reagent in column 2 of table 1 indicate the amount of vanillin present. The apparent increase in the amount of vanillin present as determined by the phenol reagent is due to the formation of vanillic acid. Since the vanillic acid formed produces more color with the phenol reagent than the vanillin which it replaced, the color developed was greater than that produced by the original amount of vanillin. The decrease in the amount of vanillin as determined by the phenol reagent which occurred after the third or fourth day is evidently due to the fact that the vanillin had been oxidized for the most part to vanillic acid, and due to its destruction the amount of vanillic acid present had begun to decrease. In other words, in the early stages of digestion vanillic acid accumulated in the culture solution, the vanillin being oxidized more rapidly than the vanillic acid was destroyed, and the accumulated vanillic acid was then destroyed.

A rough approximation from the data in table 1 can be made of the amount of vanillic acid present on the third day. In a mixture of 50 parts per million of vanillin and 225 parts per million of vanillic acid the color developed with the phenol reagent compared with that developed by a standard of 200 parts per million of vanillin was 400 parts per million. Since there were present 50 parts per million of vanillin, the vanillic acid evidently produced 350 parts per million of the color. Dividing this figure by the amount of vanillic acid present gives us the factor of 1.55. On the third day from the day of inoculation 56 parts per million of vanillin were found by the Estes reagent to be present in the culture solution. With the phenol reagent the figure was 504 parts per million. Subtracting from this figure the value found with the Estes reagent and dividing by the above factor, we have a value for the vanillic acid of 288 parts per million. This figure is greater by some 70 parts per million than is possible from the amount of vanillin which had disappeared from the culture solution. This is probably due to inaccuracies in the original determination and to the fact that the factor used was determined for a mixture which only approximated the proportions of vanillin and vanillic acid actually present in the culture solution. The results indicate, however, that little of the vanillic acid formed had been destroyed by the third day.

What products in addition to vanillic acid are formed by the action of the bacteria studied on vanillin it is impossible to state at present. The fact that a sterile solution of vanillin and mineral nutrient salts inoculated with a pure culture of the vanillin-destroying bacteria will eventually give no test with the phenol reagent of Folin and Denis indicates that the final products of the action of this organism on vanillin probably no longer possess a phenolic nature.

That the final products are not toxic to wheat has been demonstrated in an earlier publication (11).

From the chemical standpoint the oxidation of vanillin to vanillic acid by microorganisms is most interesting. Tiemann (13) attempted to prepare vanillic acid from vanillin by oxidizing the latter with many oxidizing reagents but all of these attempts were unsuccessful since he either was not able to change the vanillin at all or the oxidation proceeded too far with the formation of resinous bodies. Tiemann (14) observed, however, that if vanillin were allowed to stand in moist condition in contact with the air, it was slowly oxidized to vanillic acid. Preusse (10) observed that on feeding vanillin to dogs vanillic acid in small amount was excreted in the urine. He attempted to oxidize vanillin to guaiacol by pancreatic digestion but failed. Kotake (6) fed vanillin to dogs and found that it was oxidized to vanillic acid and excreted in the urine in the form of the paired glycuronic vanillate which he separated in the form of the barium salt. Harder (5) observed that a standard solution of vanillin in water on standing for some time gave inaccurate results with the phenol reagent, probably due to the formation of vanillic acid, and he suggested a standard prepared in approximately 20 per cent alcohol.

The demonstration that the destruction of vanillin is a biological oxidative process is of importance from the standpoint of soil fertility[6] because it indicates that the elimination of this compound in the soil is influenced by the factors which affect the supply of oxygen to the soil.

SUMMARY

1. Vanillic acid was isolated from solution cultures containing nutrient mineral salts and vanillin, and inoculated with a pure culture of a soil bacterium.

2. The melting point of vanillic acid is 210.5–211°C. (corr.).

3. Both vanillin and vanillic acid produce a blue color with the phenol reagent of Folin and Denis. Vanillic acid gives only a faint color with Estes' acid nitrate of mercury.

4. In solution cultures containing mineral nutrient salts and vanillin and inoculated with a pure culture of the vanillin-destroying bacteria, isolated from the soil, the vanillin is oxidized to vanillic acid. The vanillic acid at first accumulates in the culture medium but is later destroyed.

[6] We have found by the use of Estes' acid nitrate of mercury and the phenol reagent that vanillin is oxidized to vanillic acid in the soil as well as in solution cultures.

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THE ELECTROMETRIC TITRATION OF PLANT JUICES[1]

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The reaction of plant protoplasm is of fundamental importance in studies upon plant metabolism (1, 3, 8). Although life processes are affected primarily by the actual acidity[3] of the protoplasm; nevertheless, the total acidity may become of importance in various ways.

The actual acidity of plant juices may be determined by means of electrometric or colorimetric methods (2, 7, 9). The total acidity is usually determined by titration with standard alkali, using an indicator like phenolphthalein or litmus. This has yielded important data regarding the metabolism of succulent plants (4, 5). However, it has been observed that in some cases the natural coloring matter in plant juices makes the use of indicators difficult and the results doubtful.

In this work the desirability of having a method which is not dependent on indicators is clearly evident. It is important also to know not only the actual acidity of the extracted (6) juice, but also the actual acidity after any addition of alkali, together with the value for the total acidity corresponding to this actual acidity. Fortunately, such a method is available, but apparently has not been employed by investigators working upon plant juices. The method is that of the gas-chain, essentially of the form described by Hildebrand (8).

An inexpensive and simplified gas-chain of this type was set up. A capillary electrometer, with an improvised horizontal microscope, served as a zero instrument. A Weston voltmeter, reading directly to 0.01 volt and capable of being estimated to 0.001 volt, was used to measure the electromotive force. Several dry cells arranged in parallel and attached to a Wheatstone slidewire bridge, furnished the voltage required. The calomel cell and the electrode employed were similar to those described by Bovie (8), except that a narrow sheet of platinum was used as the electrode instead of a platinum wire. Hydrogen was obtained from a Kipp generator containing arsenic-free zinc and C.P. hydrochloric acid. The hydrogen was purified by

[1] A preliminary paper on a phase of the study of the relation of soil acidity to plants and bacteria.

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[3] By actual acidity is meant the quantity of dissociated acids present; whereas, the total acidity includes both the dissociated and the undissociated acids.

passing it through wash bottles containing successively potassium permanganate, alkaline pyrogallol (2 bottles), and alkali. The last wash-bottle was used primarily to observe and regulate the rate of flow of hydrogen. By attaching a pressure bottle to the top of the Kipp generator it was possible to secure a uniform flow of hydrogen.

The titration vessel consisted of a glass test tube (about 3 by 10 cm.) fitted with a $\frac{1}{2}$-inch thick rubber stopper. The calomel electrode and titration vessel were placed in an electrically heated water bath regulated at 25°C. The capillary connecting tube, containing KCl, and the electrode, were inserted through holes in the stopper. Two other holes were also cut in the rubber stopper—one for insertion of the burette tip (which should be long enough to extend to within about 1 inch of the surface of the plant juice), and the other a small opening for the exit of gas. Ordinarily a Bunsen valve can be used for the exit of gas, but frequently the frothing of the plant juice required the use of a glass tube blown out at one end into a small bulb punctured with fine holes. These holes can be made readily by means of a hot platinum wire. This tube was inserted through the rubber stopper, leaving the bulb in the upper portion of the titration vessel. By carefully regulating the flow of hydrogen, losses by frothing do not occur. The results obtained with the apparatus just described were frequently compared with those obtained with another gas chain in this laboratory operated by C. B. Clevenger. This was equipped with a Leeds and Northrup potentiometer, a sensitive galvanometer and electrolytically prepared hydrogen. Determinations of actual acidity, when carried out on both pieces of apparatus, were seldom more than 0.05 P_H apart and frequently differed by only 0.01 P_H. The voltmeter apparatus, as described, was found so simple, accurate and extremely rapid, that it was chosen for the titrations of the plant juices.

The juice of the plants was usually secured as follows: The tissues were thoroughly macerated and placed on a clean linen cloth; the juice was then expressed by turning the ends of the cloth in opposite directions.

Maceration by the above method was more desirable than with a mortar and pestle. The linen cloth was more convenient than a Witt laboratory press for expressing the juice. The cloth was thoroughly washed with pure water after each use. In the case of many plants it was found impossible to secure colorless solutions even by filtering, which was consequently omitted. The juice was usually centrifuged for 10 minutes to remove coarse suspended material. On standing, the acidity of some plant juices changed slightly and hence in some cases centrifuging was omitted in order to expedite the titration.

By means of a pipette a measured quantity of the juice was than transferred to the titration tube. The calomel cell connections were made and the electrode was then placed in the titration tube. It was found advantageous to have the electrode tube permanently held at the desired height in

the stopper of the titration tube. The hydrogen was then bubbled through the plant juices, and the actual acidity P_H, was determined in the usual manner. The burette tip was then inserted through the rubber stopper and a measured quantity of alkali added. After thoroughly mixing the solution by means of the bubbling hydrogen, a second reading of the actual acidity P_H was made. It was found that considerable time could be saved by bubbling hydrogen at first quite rapidly through the solution for a few moments and then very slowly; so that the bubbling of the gas was scarcely perceptible. Equilibrium was then rapidly attained. Successive additions of alkali were similarly made and the actual acidity P_H corresponding to each new equilibrium was obtained.

As examples of such determinations [4] the data for soybeans and rhubarb are given. Medium yellow soybeans were planted April 17 in yellow sand to which had been added $CaCO_3$, $(NH_4)_2SO_4$, KH_2PO_4, $Fe_3(PO_4)_2$, $MgSO_4$, and nodule-bacteria. The tops of the plants were cut May 28, the juice was extracted and centrifuged for 15 minutes and finally 10 cc. of the juice was run into the titration tube. The data obtained are as follows:

ACTUAL ACIDITY	0.0684 N NaOH ADDED
P_H	*cc.*
5.85	0
6.04	1.0
6.28	2.0
6.49	3.0
6.70	4.0
6.88	5.0
7.05	6.0
7.29	7.0
7.72	8.0
8.05	9.0
8.22	10.0
8.54	11.0
8.82	12.0
9.20	13.0

A rhubarb stalk (with leafblade removed) was taken directly from the field and the juice extracted and centrifuged for 10 minutes. The values obtained when 8 cc. of juice was used, are as follows:

[4] These examples in which soybean tops are compared with rhubarb stalks are not intended for critical comparison; since the writer has found, as will be indicated in subsequent papers, that the acidity of the leaves, stems and roots of the same plant may differ quite markedly in their actual and total acidities.

ACTUAL ACIDITY	0.0684 N NaOH ADDED
P_H	cc.
3.36	0
3.62	1.85
3.83	4.85
4.35	8.95
4.79	12.95
5.09	14.85
8.41	18.95
9.27	19.35
10.00	19.85
10.63	20.95

Curves are given in figure 1, showing the actual acidity P_H against the cubic centimeters of standard alkali added.

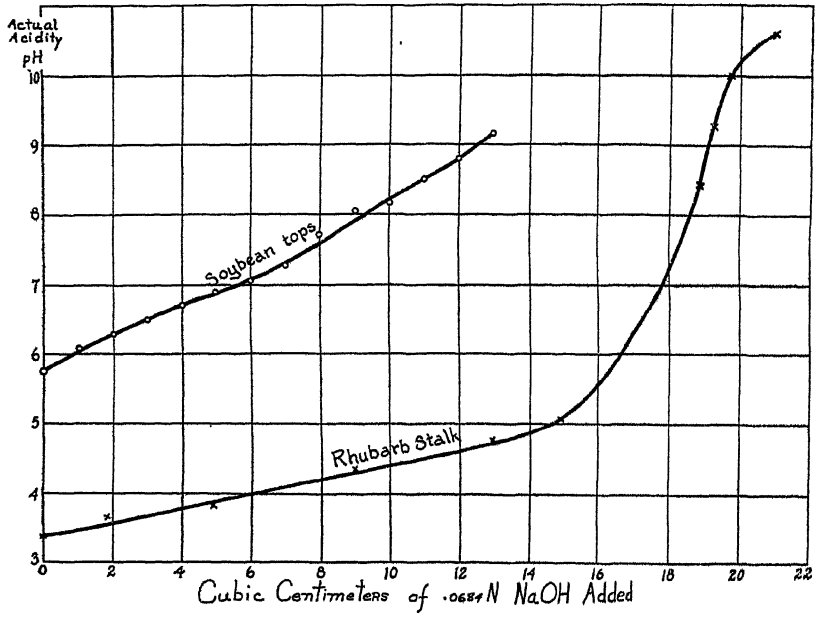

FIG. 1. CURVES SHOWING THE RELATION OF ACTUAL ACIDITY P_H WITH THE AMOUNT OF STANDARD ALKALI ADDED, FOR THE SOYBEAN AND RHUBARB

It is evident that if the P_H values at which indicators change color are known, it is possible to tell from the curves the quantity of alkali required to bring the plant juice to the turning point for any indicator. For example, in the curve for soybean tops, since the turning point of litmus is at P_H 6.83, it is evident that 4.9 cc. of 0.0684 N NaOH must be added to the 10 cc. of juice to reach the turning point for litmus. If we choose phenolphthalein as

the indicator (with its color change at P_H 8.3) it is seen from the curve that 10.3 cc. of 0.0684 N NaOH are required in order to bring the 10 cc. of juice to the turning point of phenolphthalein. By reducing the quantity of alkali added each time, it is possible to define the curves more accurately. It is obvious that the curves show the presence of greater quantities of buffer substances in the juice of the rhubarb than in the juice of soybean tops. The actual acidity of rhubarb juice is seen to be much greater than that of soybean tops. The curve for rhubarb rises very gradually at first, but once the buffer substances have been used up, the curve becomes quite abrupt.

Obviously, by titrating with acid instead of alkali, it is possible to prolong the lower ends of the curves. These methods of attack open a fertile field of study. It includes the determination of the buffer processes, the acid and the alkali reserve and the actual and total acidities of plant juices. Furthermore, the method makes possible a study of some of the relations of the nutrients in the soil upon the actual and total acidities of plant juices and the relations of the symbiotic bacteria to these.

Detailed studies of these and other phases of the reaction of plant juices are in progress.

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